Chapter 4: Sociocultural Issues in Menopause

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Cross-cultural comparisons demonstrate that reported symptoms can vary significantly among countries and among ethnic groups within countries.

Key Points[a]

1. Attitudes toward and beliefs about menopause vary historically and among cultures [C].
2. Cross-cultural comparisons demonstrate that reported symptoms can vary significantly among countries and among ethnic groups within countries in their type (e.g., vasomotor, psychological) and in the degree of distress caused [C].
3. Difficulties in integrating findings from cross-cultural studies stem from a number of limitations. Among these are differences among cultures in language used to describe symptoms; use of different methodologies in study design and in instruments used to measure symptoms; and differences in diet and other lifestyle factors that make it difficult to establish cultural versus biologic causes of symptom expression.
4. A better appreciation of cross-cultural differences in the experience of menopause may derive from an emerging interdisciplinary model in which symptoms are seen as a result of increased vulnerability due to hormonal changes in interaction with psychological and sociocultural factors.

1. The Meaning of Menopause

The sociocultural aspects of menopause have not been the focus of attention or research interest to the same extent as menopause as a physiological event. Historically and cross-culturally, perspectives on menopause have varied widely. The 19th-century Victorian image was an aging woman with a decaying body, prone to illness and insanity.[1] In contrast, the view of menopause among Asian women has focused on freedom from pregnancy and a sense of liberation.[2] Bowles emphasized that women's experience of and attitude toward menopause are influenced by beliefs and expectations inherent in the prevailing sociocultural paradigm.[3] Thus, factors such as cultural beliefs, values, and attitudes toward menopause determine the experience of individual women of that stage of life as negative and troublesome or positive and liberating.

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[a] Evidence categories are given in square brackets. A = randomized controlled trials (rich body of data); B = randomized controlled trials (limited data); C = nonrandomized trials and observational epidemiologic studies; D = Panel expert judgment. (See also table 1–1.)

Hällström wrote that the psychological significance ascribed to menopause in Western countries should be regarded as a sociocultural myth.[4] By generating expectations in women, the myth can act as a self-fulfilling negative prophecy. Reproduction and child-rearing have been the primary roles defined for women. Osofsky and Seidenberg argued that many of those writing about the psychology of menopause assumed, on the basis of their own cultural biases, that women derive greater meaning from and place greater significance on their reproductive capacity than do men.[5] Barnett and Baruch emphasized the need to revise our view of women's roles, including broadening our concept of midlife by taking into account women's roles in the workplace as well as the social contexts within which they live.[6]

Menopause research needs multidisciplinary teams to address its different aspects.

Research on menopause has long been polarized. The medical model of menopause has focused on identifying symptoms of the climacteric syndrome. Endocrinologists have defined menopause as a deficiency disease requiring treatment, with symptoms believed to be directly linked to the lack of estrogen. Social scientists, on the other hand, have emphasized the social and cultural construct of menopause, holding that whether and how climacteric symptoms are experienced is influenced by that meaning. There is evidence, for instance, that negative attitudes and beliefs before menopause may predict depressed mood or other symptoms at menopause.[7,8]

Recent trends in epidemiological research have highlighted the need to integrate these opposing views into an interactive model. Flint suggested a psycho-bio-cultural model of menopause for interdisciplinary research and for a better understanding of the different aspects of women's health.[9] It is being recognized that menopause research needs multidisciplinary teams to address its different aspects. Research from disciplines such as gynecology, endocrinology, neurology, psychiatry, psychology, and anthropology may be integrated to characterize what changes menopause entails and the individual and cultural differences that occur as a result. A central question to be addressed is whether menopause is universally associated with similar physical changes and symptoms or whether there is, indeed, cross-cultural variation. Evidence of cultural diversity in perception of symptoms lends support to the hypothesis that how menopause is experienced is not ubiquitous but distinct according to cultural groups.

It may be difficult to compare the results from different studies because of methodological problems associated with the research. (See also ch. 3, sec. 3.) Many studies have used less than ideal research designs, unvalidated rating scales that have been translated from one language to another, and small clinical samples. Obermeyer et al. in a recent review[10] pointed out shortcomings of many of the studies used for cross-cultural comparisons. She emphasized the need for studies using a longitudinal design, standardized questionnaires, and an agreed-upon definition of menopause, criteria that most of the cross-cultural comparison studies do not fulfill.

2. Cross-Cultural Comparisons

Cross-cultural comparative data can help clarify the extent to which the experience of menopause is universal, provide information about symptom variability, and identify important factors influencing symptoms. Prevalence comparisons among countries of somatic symptoms of menopause, such as hot flushes, and of psychological symptoms, such as depression and changes in sexual interest, show considerable, though not always significant, differences. Among the major findings, one of the best known is the marked contrast between the relatively high prevalence of vasomotor symptoms reported in North American and

European women and the low prevalence reported by Asian women.

Lock's classic studies of Japanese perimenopausal women[11–13] have been widely cited. She collected data on these women using a self-administered questionnaire. The study was carried out in southern Kyoto, where the women were employed in factory or other blue-collar jobs; in Nagano, a rural area where the women worked on farms; and in the suburban area of Kobe, where most of the women were homemakers. The prevalence of hot flushes was low: 3 percent of the homemakers and 10 percent of the working women reported them. The Japanese women more often reported shoulder stiffness, headache, and, to a lesser extent, lumbago, symptoms not specifically linked to the menopausal state. The most striking result was that for most of the symptoms in the symptom list about 85 percent of the women gave a negative response.

A comparison of U.S., Canadian, and Japanese women by Avis et al. showed the Japanese women to have the lowest prevalence of hot flushes and of depression as well as the lowest intake of medication.[14] Such findings may lead us to believe that Asian women do not experience the intense symptoms reported by North American, European, and African women. With regard to hot flushes, however, Lock found that the Japanese women did not have a word for the concept, which had to be explained using different words.[12]

Lock's interpretation of these findings was that Japanese women do not think the same way about menopause as, for example, women in the United States. The Japanese word for midlife transition, konenki, has a social rather than biologic connotation. According to Lock, middle age in Japan is thought of foremost as a social process; the biologic changes are generally viewed as playing only a small part. Konenki is seen as a "luxury disease" suffered only by those women who have too little to do. The strong work ethic in Japanese society coupled with a certain moralistic attitude may influence women and make them less likely to complain of any physical discomfort associated with konenki. Lock also noted that women are explicitly encouraged by the government to provide nursing care for elderly relatives, since there is a marked shortage of programs to care for the aged population. Only recently women's groups have begun to debate issues associated with konenki and have argued that middle-aged women ought to focus more on their own health. Among these activists is Albery, who challenged the belief that Japanese women do not suffer from hot flushes.[15] She also advocated a more evidence-based approach to the understanding of female aging as well as endorsement by the government for gynecologists to treat menopausal symptoms using HRT.

Thus, it may be premature to accentuate social factors in the interpretation of Lock's findings. Biologic factors may have an important role. Recent research has shed light on the role of dietary factors. In much of Asia, particularly Japan, the diet is rich in phytoestrogens. Several studies have found a decrease in the frequency of hot flushes frequency in postmenopausal women in response to soy protein supplementation.[16,17] Larger, well-controlled clinical trials are needed, however, to better address the positive and possible negative effects associated with the intake of soy protein.[18,19] (See ch. 3, sec. 6.2.)

Is it possible that Asian lifestyle could have such dramatic effects on women's health? More recent studies in Asia present a somewhat different picture of symptom reporting. Among them is a well-designed, large-scale study by Boulet et al., conducted in Hong Kong, Indonesia, Korea, Malaysia, the Philippines, Singapore, and Taiwan.[20] The climacteric syndrome was, indeed, experienced by the participants, although in a milder form than generally reported in Western countries. The prevalence of hot flushes and of sweating was lower than in Western countries but not negligible. The percentages of women reporting more psychological types of complaint were similar to those in Western countries. Perhaps, as the authors suggested, distress related to vasomotor symptoms is translated into psychological complaints, which are more frequently considered to warrant consulting a physician.

Other studies of Asian women that compared symptom reporting include a cross-sectional study of Thai women in Bangkok.[21] The women were asked to report symptoms in the prior 2 weeks. They did experience hot flushes, although the most common complaints were dizziness, headache, joint pain, and backache. The sample was not representative, and the conclusions must be considered tentative, because the women were recruited as they accompanied family members or friends to the hospital. In a study of Thai women attending health clinics in Bangkok, 22 percent of the women with irregular menses and 7 percent of the postmenopausal women reported hot flushes, although the most common symptoms were dizziness (45 percent) and irritability (41 percent).[22]

Among perimenopausal Chinese women living in Hong Kong, 20 percent of those surveyed experienced hot flushes, and, again, psychological complaints such as anxiety and nervousness were more prevalent.[23] In another study of urban women, perimenopausal Canadian and Chinese women differed markedly in symptom prevalence: 60 percent and 18 percent, respectively, reported vasomotor symptoms, and the Chinese women ranked other symptoms as more important, including boredom, poor memory, numbness in the hands or feet, change in appearance, and change in ability to see, taste, or smell.[24] Both groups in the study reported sleep-related problems and fatigue. Ho et al. studied perimenopausal Hong Kong Chinese women and found that although 10 percent experienced hot flushes, musculoskeletal complaints were the most prevalent.[25]

Thus, the prevalence of hot flushes appears to be lower in Asian women, although some of the more recent studies have shown rates closer to western figures. Also, the types of symptoms reported and the degree of distress caused are often different. There are no good explanations for the observed differences. Hot flushes were previously thought to be linked directly only to estrogen deficiency and not modulated by other factors. Our study[26] and that of Avis et al.[27] showed that hot flushes are modulated by psychosocial factors, such as satisfaction with work role and stress at work. In an ongoing study, we found that women with high-stress jobs report the most frequent hot flushes (Collins A and Ahs A, unpublished results). Many of the assumed truths about climacteric symptoms may have to be modified. Because all symptoms are individual and modulated by cognitive processes to a certain extent,[28] they can be influenced by cultural factors. A clear, and perhaps related, example of such influences is the cultural variation in pain perception.[29]

There are probably important factors within cultures that can mediate symptom experience. They include differences in expectations and attitudes toward aging and menopause, as well as socioeconomic factors and women's roles and opportunities in society. A very important area of research that has been largely neglected until recently is the study of different ethnic groups within Western countries. Our ongoing population-based longitudinal study has shown that among perimenopausal women residing in the Stockholm area, women born outside Scandinavia report more frequent hot flushes than do Swedish-born women (Collins A and Ahs A, unpublished data). Researchers in the United States found that African-American women were significantly more likely than white women to report hot flushes.[30] The difference remained after adjustment for BMI, educational level, and menstrual and gynecologic history. The authors attributed the difference mainly to psychosocial factors and stress. Despite the high prevalence of symptoms, few African-American women had discussed menopausal management with their physicians.[30] A study[31] of women of the Indian subcontinent living in the United Kingdom showed that the majority regarded menopause as a natural event. However, only 33 percent were happy about menopause, and 46 percent were worried about possible adverse effects, such as ill health or weight gain. Over 75 percent of the women stated that they would like to seek medical advice about management of menopause. They also stated that they would prefer a female doctor who would be able to communicate with them in their own language. Thus, the overall results suggest a great need for information and education.

The large-scale, population-based SWAN in the United States compared symptom reporting among white, African-American, Chinese-American, and Japanese-American women.[32] The Asian-American women had significantly fewer symptoms than the white women, and the African-American women had the highest prevalence of vasomotor symptoms.[32] At the same time, among premenopausal through postmenopausal women, attitudes toward menopause and aging were found to differ among the ethnic groups; African-American women were the most positive, and Chinese- or Japanese-speaking women who received their schooling outside the United States were the least positive.[33] Menopause was described as a natural transition of life by Chinese-American and Chinese women in the United States.[34] It is important to consider where women obtain their information about menopause. Study results suggest distinct differences among ethnic groups. Several U.S. studies showed that, in general, African-American women and in particular less educated African-American women have less knowledge of menopause, are less likely to discuss menopause with a physician, and have less awareness of and less knowledge about HRT,[35–38] although they are more likely to have had a hysterectomy.[39] There can be distinct biases in prescribing HRT, and African-American women are less likely than white women to use it.[40] The findings should be related to those of a recent ethnographic study by Agee of African-American and Euro-American women of menopausal age.[41] The women were interviewed in depth about the transfer of knowledge about menopause and aging from mother to daughter. The African-American women more than the white women recounted that their mothers had provided them with the knowledge and tools to negotiate difficulties associated with menopause. They relied more on their own capacity to cope with problems encountered during the menopausal transition, and they were more prone to resist a biomedical model and, thus, less willing to follow their doctors' suggestions to start hormonal treatment. Many Euro-American women stated that their mothers had not talked about their menopausal problems, and many felt that their own life experiences set them apart from their mothers. The relative lack of a role model made them more dependent on a medical approach to solving problems related to menopause.

Also, the age at natural menopause differs between white and African-American women; the African-American women reached menopause significantly earlier.[42] In trying to explain the reasons for these differences, the authors focused on marginalization and psychosocial stress as the most significant predictors of earlier menopause among African-American women.

3. Ethnographic Studies

The degree of development of a society may also be important, and we may have to look more closely at nonwestern societies that are not as developed as those of Asia. There are unique studies of populations of rural women with a low level of formal education, and living simple lives, who show no signs of distress at menopause. Data collection was adapted to the women's lifestyle and adjusted for traditional expectancies. The findings may provide us with important indications of the role of cultural norms in symptom experience and reporting.

Beyene used a systematic ethnographic approach to collect data on 107 rural Mayan women aged 33 through 57 years.[43] The women lived in southeastern Yucatán, Mexico, where the residents were subsistence farmers practicing traditional Mayan ceremonies. The onset of natural menopause was earlier than in developed Western countries. The Mayan women became menopausal at ages 41 through 45.

The women did not consider menopause a major crisis. In general, they reported looking forward to menopause and likened it to being young and free. Menopause in the rural Mayan culture was largely unrecognized except as marking the end of menstruation and childbearing. The women indicated that the only recognized symptom of menopause was menstrual irregularity followed by the final cessation of menses. None reported hot flushes or cold sweats. The premenopausal Mayan women did not seem to have cultural knowledge or expectations relating to the onset of menopause other than the cessation of menstruation. Mayan women considered menopause to be a life stage free from taboos and restrictions. They reported better sexual relationships with their husbands, because of no risk of pregnancy.

The findings were particularly valuable because of another study's analysis of the hormonal profiles of rural Mayan women.[44] It was hypothesized that their menopause would be endocrinologically distinct. Determination of FSH, estradiol, prolactin (PRL), androstenedione, and testosterone values as well as BMD of 52 postmenopausal and 26 premenopausal rural Mayan women showed the same endocrinological profile as in U.S. women. Even with specific questioning through a native interviewer, however, it was not possible to elicit familiarity with hot flushes.[44] Interestingly, some Mayans who had moved to a nearby city experienced hot flushes.

Beyene also studied rural Greek women.[43] Data were collected from women living in a village in the eastern part of the island of Evia. The villagers were farmers using traditional farming methods, including plowing with horses and mules. Like the Mayans, the Greek women experienced menopause as a life stage free from taboos and restrictions. They, too, reported better sexual relationships with their husbands. They reported that, without risk for pregnancy, they felt more relaxed about sex. However, women also associated menopause with growing old, lack of energy, and a generally downhill course. Premenopausal women reported anxiety, negative attitudes, or anticipation, with some mixed feelings. When asked about experience of menopausal symptoms, 73 percent of the menopausal and postmenopausal women reported having had hot flushes, and 30 percent reported having cold sweats. Unlike Mayans, Greek women understood the concept of hot flush, and the older women even offered the Greek word for this symptom. They were also able to give detailed accounts of the process of hot flushes and the times they most often felt the sensations and changes in their bodies. Women said they experienced more hot flushes at night and around the times they usually expected to have their menstrual periods.

These two cultures are very different from the other cultures studied, since these are rural women living in a village, where the form of life is still very traditional. In both of them, the women were farmers with physically strenuous work. Possible explanations for reduced symptomatology in the rural groups could relate to physical exercise[45] or dietary habits, although studies of such lifestyle components have yielded mixed results. (See ch. 3, sec. 5.1.) Social or socioeconomic status probably plays an important part. Not all women have access to modern medical care. In an interview study of women with spontaneous menopause in Karachi, Pakistan, 6 percent of slum dwellers sought treatment for symptoms, compared with 26 percent of middle class clinic attendees and 38 percent of the most privileged group, wives of retired military officers.[46] Only one-fifth of the slum dwellers reported symptoms, whereas 57 percent of the middle class and 50 percent of the most privileged group reported hot flushes. In a study in India, women living in a culture in which social status increased with age experienced few symptoms.[47]

A study of menopausal Nigerian women of Yoruba descent found 30 percent to have had hot flushes.[48] Joint pain was the most frequently reported symptom. The authors suggested that the Yoruba women may not have been as aware of hot flushes as white women and that they may have wrongly attributed their sensations to environmental temperature or to fever.

In an ongoing population-based study of Arab women, Obermeyer reported on the occurrence of symptoms and on help seeking by perimenopausal women in Beirut, Lebanon.[49] The proportion of women reporting hot flushes was 45 percent, similar to figures in the United States, Canada, and Sweden. Reported depression was similar to that in Canada but lower than the U.S. figure of 36 percent. The frequency of hot flushes was higher in smokers, and women who were employed reported fewer symptoms. Thirty-nine percent had sought help for their symptoms, and 15 percent reported using HRT, figures the authors interpreted as consistent with the high educational level of Beirut women and the degree of medicalization in the country.

4. What Can Cross-Cultural Comparisons Tell Us?

Flint and Samil[50] and Obermeyer et al.[10] in reviews of the literature emphasized the need for integration of the biomedical and developmental views of menopause.

Attempts to verify menopausal symptoms in different cultures have proved difficult. (See ch. 3.) Results from different studies are hard to compare because the quality of data can differ and studies differ in design and subject representativeness. Often, the subjects were patients or volunteers. Much research has used rating scales translated from European or North American studies; concerns have been raised about the appropriateness of translations without consideration of the cultural relevance of the questionnaires' content. Many studies cited above demonstrated that symptom types and patterns vary from country to country. Vasomotor symptoms are the most frequently reported symptoms in Europe and North America, but in Asia psychological complaints appear to be more common. Yoruba women in Nigeria described joint pain most commonly. The interpretation of bodily states and, thus, symptom experience may be different in different cultures. Boulet et al. suggested that Asian women may report vasomotor symptoms less frequently because vasomotor distress is experienced more in psychological terms.[20] There is a need to know how to ask the right questions and a need for more knowledge of the cultures being studied. In addition, more reliable scales are needed, and these have to be developed in a cultural context.

Overall, there is an association between hormonal changes and climacteric symptomatology, and the association is modulated by cultural factors. There is a considerable variation in the prevalence and pattern of symptoms in different countries, a variation probably due to diversity in cultural norms and traditions as well as in diet and other lifestyle factors.

5. Access to Health Care

Kaufert developed a model of menopause in which there are important social implications of becoming menopausal that vary from one society to another.[51] The definition of menopause for any culture will be derived from the meaning and consequences of menopause and from how women's roles are defined in that society. Women are aware of these stereotypes and interpret their bodily changes in accordance with what they have learned. The experience of menopause is associated with a woman's health history as well as a wide range of variables, such as genetic factors, diet, education, marital status, number of pregnancies, the kind of work she has carried out, social support, and access to health care.[52]

Women need education and balanced information to make personal decisions regarding whether to use HRT.

Access to health care varies widely among countries. Women's choices around menopause are said to bear consequences for their health in old age. For a long time, hot flushes and night sweats were considered to be core symptoms of menopause and the most important reason for using HRT. More recently, HRT has been widely promoted for prevention against osteoporosis, CVD, and an array of other conditions. Educated women in industrialized countries with well-developed medical care are a privileged group with access to the most recent information on HRT, whereas other groups have less access and less knowledge. On the other hand, medicalization of menopause in cultures in which menopause is not perceived as a problem is an important issue that should be debated. Some critics have questioned the role of the pharmaceutical industry in influencing health care through product promotion.[53] Developing countries with scarce resources are less likely than developed countries to allocate funds for care of menopausal women. In addition, the inequality among social groups within countries can result in very different access to information and care.

Promoting positive attitudes to aging and to menopause could be important in modifying symptoms and improving the health of women.[54] Women need education and balanced information to make personal decisions regarding whether to use HRT. An important goal for health care providers should be to educate women. Such education should lead to greater equality among women in different cultures and social levels and help women control their own health.

6. Conclusions

Menopause has long been considered a turning point in women's lives in western cultures. Although menopause as a physiologic event remains constant, attitudes toward and beliefs about menopause vary considerably historically and cross-culturally. In the past decade, there has been a heated debate among biomedical and social scientists as to whether menopause should be seen as a deficiency disease rather than a natural event. Cross-cultural comparisons fuel the debate by showing that the relation between hormones and symptoms is, indeed, complex. There are significant differences in patterns and prevalence of symptoms between countries and, interestingly, in the types of symptom reported in different ethnic groups within countries. It is difficult, however, to draw firm conclusions from available cultural and ethnographic comparison studies because of a number of limitations. Among these are differences among cultures in language used to describe symptoms and in women's inclination to report symptoms; use of different methodologies in study design and instruments used to measure symptoms; and differences in diet and other lifestyle factors that make it difficult to establish cultural versus biologic reasons for symptom expression. (See ch. 3.)

7. Future Needs

- As cross-cultural research on menopause has been hampered by methodological difficulties, better controlled, population-based studies are needed which use standardized instruments adapted to the culture studied.
- There is growing recognition that investigators in different disciplines have to work together for a better understanding of women's health at menopause; such collaboration is particularly needed for cross-cultural work.
- An interactive psycho-bio-cultural model of menopause is needed, which recognizes the interplay between the individual and her psychosocial and cultural environment. From such a perspective, symptoms can be seen as the result of increased vulnerability due to hormonal changes interacting with psychological and sociocultural factors.
- Access to health care has been shown to vary among countries and among socioeconomic groups within countries. It is important that research results be disseminated within the cultures under study so that women can make their personal decisions about possible interventions and treatment strategies.

REFERENCES

[1] Gannon L, Ekstrom B. Attitudes toward menopause—the influence of sociocultural paradigms. *Psychology of Women Quarterly* 1993;17:275–88.

[2] Maoz B, Dowty N, Antonovsky A, Wijsenbeek H. Female attitudes to menopause. *Soc Psychiatry* 1970;5:35–40.

[3] Bowles CL. The menopausal experience: sociocultural influences and theoretical models. In: Formanek R, ed. The meanings of menopause: historical, medical, and clinical perspectives. Hillsdale, NJ: Analytic Press, 1990;157–75.

[4] Hällstrom T. Mental disorder and sexuality in the climacteric. Copenhagen: Scandinavian University Books, 1973.

[5] Osofsky HJ, Seidenberg R. Is female menopausal depression inevitable? *Obstet Gynecol* 1970;36(4):611–5.

[6] Barnett RC, Baruch GK. Social roles, gender, and psychological distress. In: Barnett RC, Biener L, Baruch GK, eds. Gender and stress. New York: Free Press, 1987;122–43.

[7] Hunter MS. Psychological and somatic experience of the menopause: a prospective study [published erratum appears in *Psychosom Med* 1990;52(4):410]. *Psychosom Med* 1990;52(3):357–67.

[8] Olofsson ASB, Collins A. Psychosocial factors, attitude to menopause and symptoms in Swedish perimenopausal women. *Climacteric* 2000;3:33–42.

[9] Flint M. Menopause—the global aspect. In: Hammar M, Berg G, eds. The modern management of the menopause: A perspective for the 21st century. The International Congress, Symposium, and Seminar Series, vol. 8. Proceedings of the Seventh International Congress on the Menopause, Stockholm, Sweden, June 1993. New York: Parthenon, 1994;17–22.

[10] Obermeyer CM, Ghorayeb F, Reynolds R. Symptom reporting around the menopause in Beirut, Lebanon. *Maturitas* 1999;33(3):249–58.

[11] Lock M. Ambiguities of aging: Japanese experience and perceptions of menopause. *Cult Med Psychiatry* 1986;10(1):23–46.

[12] Lock MM. Encounters with aging: mythologies of menopause in Japan and North America. Berkeley: University of California Press, 1993.

[13] Lock M. Menopause: lessons from anthropology (review). *Psychosom Med* 1998;60(4):410–9.

[14] Avis NE, Kaufert PA, Lock M, McKinlay SM, Vass K. The evolution of menopausal symptoms. *Baillieres Clin Endocrinol Metab* 1993;7(1):17–32.

[15] Albery N. The menopause in Japan — Konenki Jigoku (editorial) *Climacteric* 1999;2:160-1.

[16] Fitzpatrick LA. Selective estrogen receptor modulators and phytoestrogens: new therapies for the postmenopausal women (review). *Mayo Clin Proc* 1999;74(6):601–7.

[17] Albertazzi P, Pansini F, Bonaccorsi G, Zanotti L, Forini E, De Aloysio D. The effect of dietary soy supplementation on hot flushes. *Obstet Gynecol* 1998;91(1):6–11.

[18] Finkel E. Phyto-oestrogens: the way to postmenopausal health? *Lancet* 1998;352(9142):1762.

[19] Eden J. Phytoestrogens and the menopause (review). *Baillieres Clin Endocrinol Metab* 1998;12(4):581–7.

[20] Boulet MJ, Oddens BJ, Lehert P, Vemer HM., Visser A. Climacteric and menopause in seven South-east Asian countries. *Maturitas* 1994;19(3):157–76.

[21] Punyahotra S, Dennerstein L, Lehert P. Menopausal experiences of Thai women. Part 1: Symptoms and their correlates. *Maturitas* 1997;26(1):1–7.

[22] Chompootweep S, Tankeyoon M, Yamarat K, Poomsuwan P, Dusitsin N. The menopausal age and climacteric complaints in Thai women in Bangkok. *Maturitas* 1993;17(1):63–71.

[23] Tang GWK. Menopausal symptoms. *Journal of the Hong Kong Medical Association* 1993;45:249–54.

[24] Hilditch JR, Chen S, Norton PG, Lewis J. Experience of menopausal symptoms by Chinese and Canadian women. *Climacteric* 1999;2:164–73.

[25] Ho SC, Chan SG, Yip YB, Cheng A, Yi Q, Chan C. Menopausal symptoms and symptom clustering in Chinese women. *Maturitas* 1999;33(3):219–27.

[26] Collins A. Psychological aspects of the transition to menopause. In: Aso T, ed. The Menopause in the new millennium. Lancs, United Kingdom: Parthenon, 2000.

[27] Avis NE, Crawford SL, McKinlay SM. Psychosocial, behavioral, and health factors related to menopause symptomatology. *Womens Health* 1997;3(2):103–20.

[28] Pennebaker JW, Epstein D. Implicit psychophysiology: effects of common beliefs and idiosyncratic physiological responses on symptom reporting. *J Pers* 1983;51(3):468–96.

[29] Bates MS, Edwards WT, Anderson KO. Ethnocultural influences on variation in chronic pain perception. *Pain* 1993;52(1):101–12.

[30] Grisso JA, Freeman EW, Maurin E, Garcia-Espana B, Berlin JA. Racial differences in menopause information and the experience of hot flashes. *J Gen Intern Med* 1999;14:98–103.

[31] Sethi K, Pitkin J. British-Asian women's views on and attitudes towards menopause and hormone replacement therapy. *Climacteric* 2000;3:248–53.

[32] Avis NE, Stellato R, Crawford S, Bromberger J, Ganz P, Cain V, Kagawa-Singer M. Is there a menopausal syndrome? Menopausal status and symptoms across racial/ethnic groups. *Soc Sci Med* 2001;52(3):345–56.

[33] Sommer B, Avis N, Meyer P, et al. Attitudes toward menopause and aging across ethnic/racial groups [published erratum appears in Psychsom Med 2000;62:96]. *Psychosom Med* 1999;61:868–75.

[34] Adler SR, Fosket JR, Kagawa-Singer M, et al. Conceptualizing menopause and midlife: Chinese American and Chinese women in the US. *Maturitas* 2000;35(1):11–23.

[35] Brown AF, Perez-Stable EJ, Whitaker EE, et al. Ethnic differences in hormone replacement prescribing patterns. *J Gen Intern Med* 1999;14(11):663–9.

[36] Appling SE, Allen JK, Van Zandt S, Olsen S, Brager R, Hallerdin J. Knowledge of menopause and hormone replacement therapy use in low-income urban women. *J Womens Health Gend Based Med* 2000;9(1):57–64.

[37] Domm JA, Parker EE, Reed GW, German DC, Eisenberg E. Factors affecting access to menopause information. *Menopause* 2000;7(1):62-7.

[38] MacDougall LA, Barzilay JI, Helmick CG. Hormone replacement therapy awareness in a biracial cohort of women aged 50-54 years. *Menopause* 1999;6(3):251–6.

[39] Meilahn EN, Matthews KA, Egeland G, Kelsey SF. Characteristics of women with hysterectomy. *Maturitas* 1989;11(4):319–29.

[40] Bartman BA, Moy E. Racial differences in estrogen use among middle-aged and older women. *Womens Health Issues* 1998;8(1):32–44.

[41] Agee E. Menopause and the transmission of women's knowledge: African American and white women's perspectives. *Med Anthropol Q* 2000;14(1):73–95.

[42] Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prospective study of the determinants of age at menopause. *Am J Epidemiol* 1997;145:124–133.

[43] Beyene Y. Cultural significance and physiological manifestations of menopause. A biocultural analysis. *Cult Med Psychiatry* 1986;10:47–71.

[44] Martin MC, Block JE, Sanchez SD, Arnaud CD, Beyene Y. Menopause without symptoms: the endocrinology of menopause among rural Mayan Indians. *Am J Obstet Gynecol*, 1993;168(6 Pt 1):1839–45.

[45] Hammar M, Berg G, Lindgren R. Does physical exercise influence the frequency of postmenopausal hot flushes? *Acta Obstet Gynecol* Scand 1990;69(5):409–12.

[46] Wasti S, Robinson SC, Akhtar Y, Khan S, Badaruddin N. Characteristics of menopause in three socioeconomic urban groups in Karachi, Pakistan. *Maturitas* 1993;16(1):61–9.

[47] George T. Women in a south Indian fishing village: role identity, continuity, and the experience of menopause. *Health Care Women Int* 1996;17(4):271–9.

[48] Okonofua FE, Lawal A, Bamgbose JK. Features of menopause and menopausal age in Nigerian women. *Int J Gynaecol Obstet* 1990;31(4):341–5.

[49] Obermeyer CM. Menopause across cultures: a review of the evidence (review). *Menopause* 2000;7(3):184–92.

[50] Flint M, Samil RS. Cultural and subcultural meanings of the menopause (review). *Ann N Y Acad Sci* 1990;592:134–48.

[51] Kaufert PA. Myth and the menopause. *Sociol Health Illn* 1982;4:141–66.

[52] Kaufert PA. The social and cultural context of menopause (review). *Maturitas* 1996;23(2):169–80.

[53] Coney S, Seaman B. The menopause industry: how the medical establishment exploits women. Alameda, CA: Hunter House, 1994.

[54] Hemminki E, Topo P, Kangas I. Experience and opinions of climacterium by Finnish women. *Eur J Obstet Gynecol Reprod Biol* 1995;62(1):81–7.

Chapter 5: Physiological Role of Estrogen and Estrogen Receptors

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There are two different ER subtypes, ERα and ERβ, that mediate the biological effects of estrogens and antiestrogens. Different ligands induce different ER conformations.

Key Points[a]

1. There are two different ER subtypes, ERα and ERβ, that mediate the biological effects of estrogens and antiestrogens. Different ligands induce different ER conformations.
2. Different mechanisms of target gene regulation affect the agonist-antagonist profile of a ligand. SERMs have a tissue- and gene-specific mixed agonist-antagonist effect.
3. Both ERα and ERβ are expressed in human breast cancer. Measurement of both ERα and ERβ is suggested for selection of appropriate breast cancer therapy.
4. Both ERα and ERβ are important for normal ovarian follicular development and female fertility.
5. ERβ-selective agonists may protect from abnormal prostate growth and may be the therapy of choice for urge incontinence in women.
6. Available data suggest that ERα plays an important role in bone maturation and homeostasis in both women and men but that ERβ also has a specific role in bone physiology in women.
7. ERα and ERβ are expressed in vascular endothelial cells, smooth muscle cells, and myocardial cells. Potential beneficial effects of estrogens on cardiovascular function and reactivity stem from direct effects on cells in the vascular system but also from effects on liver and circulating monocytes-macrophages.
8. Estrogens are linked to a variety of functions in the CNS: learning, memory, awareness, fine motor skills, temperature regulation, mood, reproductive functions, and depression. The predominant expression and localization of ERβ in rat neocortex, hippocampus, and nuclei of the basal forebrain suggest an important role for ERβ in learning and memory.

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[a] Evidence categories are given in square brackets. A = randomized controlled trials (rich body of data); B = randomized controlled trials (limited data); C = nonrandomized trials and observational epidemiologic studies; D = Panel expert judgment. (See also table 1–1.)

9. Estrogen and inhibins produced by the ovaries are important feedback regulators of the hypothalamo-pituitary axis (HPA) and the serum levels of LH and FSH. ERα seems to be more involved in the LH, FSH feedback loop than ERβ.

10. ERα and ERβ subtype-selective SERMs may better provide the benefits of estrogen replacement therapy (third generation HRT) than currently used treatments.

Estrogens have an important role in maintaining a balanced bone metabolism.

Extended Summary

Introduction: Nuclear receptors such as the ER are ligand-dependent transcription factors. There are two different ER subtypes, ERα and ERβ, that mediate the biological effects of estrogens and antiestrogens. ERβ exists in multiple isoforms. Different ligands induce different ER conformations, and there is a dramatic difference in the topology of the ER surface between agonist- and antagonist-bound receptor. Coactivators and corepressors interact with ligand-bound ERα and ERβ and play, together with the receptor, an important role in the regulation of ER target-gene expression. Different modes or mechanisms of target gene regulation affect the agonist-antagonist profile of a ligand. SERMs have a tissue- and gene-specific mixed agonist-antagonist effect. Alternative indirect activation pathways, other than binding of natural or synthetic small organic hormones or drugs, can also modulate the ER activity. Estrogens have also very rapid effects, so-called nongenomic effects. Nonreceptor-dependent antioxidant effects by estrogens have been reported, protecting from neurodegenerative disorders and atherogenesis.

Breast Tissue: There is no pubertal breast development in aromatase-deficient women due to lack of or too low levels of circulating estrogens. Estrogen therapy of aromatase-deficient female patients led to normal prepubertal and postpubertal breast development. ERα has been shown to be necessary for mouse mammary gland development. ERβ is abundantly expressed in rat breast. Both ERα and ERβ are present in human breast cancer. Measurement of both ERα and ERβ is suggested for selection of appropriate breast cancer therapy.

Urogenital Tract: ERα and ERβ are both expressed in uterus, ovary, testes, and prostate in the mouse. Absence of ERα results in infertility in both male and female mice. Absence of ERβ results in partial infertility in female mice but no impaired fertility in male mice. ERβ-deficient mice display hyperplasia, dysplasia, and prostatic intraepithelial neoplasia (PIN)-lesions of the prostate. Deficiency of aromatase in human females led to ambiguous genitalia and polycystic ovaries. ERT of aromatase-deficient female patients led to resolution of the ovarian cysts and menarche. Male patients with estrogen deficiency or estrogen insensitivity are reported with macroorchidism or oligozoospermia. ERβ-selective agonists may protect against abnormal prostate growth and may be the therapy of choice for urge incontinence.

Bone: Estrogens have an important role in maintaining a balanced bone metabolism. In addition, estrogens protect postmenopausal women from bone loss and the development of osteoporosis. Estrogens may play an important role in the maintenance of bone mass in aging men. Estrogens are important for the pubertal growth spurt and epiphyseal closure in girls as well as in boys. There are likely both direct and indirect (systemic) effects of estrogens on bone metabolism and homeostasis. Both ERα and ERβ are expressed in the bone-forming osteoblasts. Estrogen insensitivity in a male patient caused by ERα deficiency led to osteopenia and continuous longitudinal growth due to unfused epiphyses. Male and female patients with aromatase deficiency have increased bone turnover, delayed bone maturation, low

BMD, and tall stature due to unfused epiphyses. ERT of both female and male aromatase-deficient patients resulted in growth spurt, closure of the epiphyses, and increased BMD. Lack of ERβ expression in the female ERβ-/- mice led to a masculinized bone phenotype of the long bones but no effect on the bone phenotype in male mice. Available data suggest that ERα plays an important role in bone in both men and women but that ERβ perhaps has a role in bone physiology only in women.

The Cardiovascular System: A number of gender-related cardiovascular differences have been reported, for example, (1) lower risk for young women than for young men to develop atherosclerosis and CVD, (2) higher prevalence of left ventricular hypertrophy in men than in women, (3) significantly greater intimal thickening after vascular injury in men than in women, and (4) the rapid vascular response to estrogen in women but not in men. ERα and ERβ are expressed in vascular endothelial cells, in smooth muscle cells, and in myocardial cells. Both ERα and ERβ can mediate the vascular injury response to estrogens, suppressing smooth muscle cell proliferation and intimal thickening. Estrogens have both genomic and nongenomic effects on vascular tissue. Part of the beneficial effects of estrogen on cardiovascular function and reactivity comes from liver-specific effects of estrogens on the serum lipid/cholesterol profile. ERα most likely mediates the liver-specific effects of estrogens. Also monocytes-macrophages are potential targets for the beneficial effects of estrogens on the cardiovascular system and the development of atherosclerosis.

Central Nervous System and the Hypothalamo-Pituitary Axis: Estrogens are reported to influence a variety of functions in the CNS such as learning, memory, awareness, fine motor skills, temperature regulation, mood, and reproductive functions. Estrogens are also linked to symptoms of depression and treatment of depressive illness. Different brain structures and neurotransmitter systems are involved in the different effects of estrogens. The predominant expression and localization of ERβ in rat neocortex, hippocampus, and nuclei of the basal forebrain suggest an important role for ERβ in learning and memory. Estrogen, through effects on the HPA, modulates the expression and secretion of several hormones from the anterior pituitary gland, such as LH, FSH, growth hormone (GH), and PRL. Female and male patients with aromatase-deficiency have elevated levels of LH and FSH and elevated circulating levels of androgens. Substitution with conjugated estrogens in both male and female aromatase-deficient patients resulted in normalization of gonadotropin and testosterone levels. Clinical data on a male patient with an ERα nonsense mutation also showed increased circulating LH and FSH levels despite high estrogen levels. In ERα-/- mice, the serum LH but not the FSH levels were elevated despite tenfold higher circulating levels of estrogen. Available data indicate that estrogens rather than testosterone (in both men and women) together with inhibins are the major regulators of serum gonadotropin levels and that ERα seems to be more involved in this process than ERβ.

Hormone Replacement Therapy: Traditional Alternatives and Future Perspectives: The most common regimens in use to treat symptoms of the menopause and postmenopausal health risks are 17β-estradiol, esterified estrogens, or CEEs, each in combination with a progestin, for example, MPA. The awareness of undesired effects and serious health risks (breast cancer, endometrial cancer, and venous thromboembolism) with existing HRT (first generation HRT) warrants alternatives with improved safety profile. Alternative regimens for women who do not wish to take today's first generation HRT exist. Non-ER subtype-selective SERMs (second generation

ERα and ERβ are expressed in vascular endothelial cells, smooth muscle cells and in myocardial cells.

HRT) display tissue-selective estrogen agonism. Although the most frequent and serious health risks of first generation HRT are obviated by these SERMs, they still suffer from low efficacy compared to first generation HRT, and they aggravate hot flushes. The existence of two ER subtypes, ERα and ERβ, gives the opportunity to develop ER subtype-selective ligands that will most likely better provide the benefits of ERT, with an improved therapeutic profile (third generation HRT).

1. Introduction

Around 1960, Jensen and colleagues came to the conclusion that the biological effects of estrogen had to be mediated by a receptor protein.[1] Since then, two ER subtypes have been cloned, ERα[2] and ERβ.[3] The discovery of rat ERβ was rapidly followed by the cloning of ERβ from other species[4–6] and the identification of several ERβ isoforms with: (1) extended N-termini,[7,8] (2) a variant with an 18 amino acid residue insertion into the ligand-binding domain (LBD), with altered ligand-binding characteristics,[9,10] and (3) C-terminal splice variants unable to bind ligand or activate reporter gene transcription.[11,12] Various alternatively spliced forms have been described also for ERα.[13,14] The biological and physiological significance of different isoforms of ERα and ERβ is unknown and remains to be investigated. Whether there is still another ER subtype (e.g., ERγ) to be found remains an open question. However, the vascular protective effect of estrogen in the absence of ERα and ERβ[15] and the fact that ERα and ERβ double knockout (DERKO) mice survive to adulthood[16] may suggest that yet another unidentified ER exists.

ERα and ERβ are similar in their architecture to the other members of the steroid-thyroid hormone superfamily of nuclear receptors[17,18] in that they are composed of independent but interacting functional domains: the N-terminal A/B domain, the least conserved among nuclear receptors, enables the receptor to interact with members of the transcriptional apparatus; the C domain, involved with binding of DNA, contains two zinc-binding motifs and a dimerization interface that mediates cooperativity in DNA binding; the D domain, also referred to as a "hinge region," necessary to give the receptor some flexibility between the DNA and the LBDs, binds heat shock protein hsp 90, and probably harbors the sequence representing the nuclear localization signal; and the E/F multifunctional domain recognizes and binds a ligand and is involved in receptor dimerization and interaction with transcription factors and cofactors.[19] The gene modulatory effect of a receptor following binding of a ligand depends on the conformational change of the receptor induced by the ligand and the subsequent events, including release of inhibitory proteins (heat shock proteins), receptor dimerization, receptor:DNA interaction, recruitment of and interaction with coactivators and other transcription factors, and the formation of a preinitiation complex.[20]

In particular, two regions in the ERα participate in transcriptional activation of target genes by forming protein:protein contacts with other transcription factors or coactivators, the ligand-independent, N-terminal activation function 1 (AF–1) and the ligand-dependent, C-terminal activation function 2 (AF–2).[17, 20–23] Synthetic ligands with mixed agonist-antagonist activity, so-called SERMs, such as tamoxifen and raloxifene, display a low but significant partial estrogen agonist activity via ERα on an estrogen-responsive element (ERE).[24] In contrast, these mixed agonists-antagonists display pure antagonism via ERβ on an ERE site. The partial agonism of the SERM tamoxifen by ERα is mediated by a slightly different part of the ERα AF–1 region than required for estradiol (E2) signaling.[25] The pure antagonism of tamoxifen by ERβ was recently explained by the lack in human ERβ of this particular function of hERα AF–1.[26] Thus, differences in the amino-terminal regions of ERα and ERβ most likely explain the differences in their response to mixed agonists-antagonists, such as tamoxifen and raloxifene, on an ERE site. (See also ch. 6, sec. 3.2.4 for mechanism of antagonist action.)

The ER LBD, similar to other intracellular receptors, is made up of 12 α-helices, named H1–H12. Helix 12 (H12), together with amino acid residues in helices H3, H4, and H5, constitute the AF–2 coactivator recruitment and interaction surface.[20,27] The resolution of 3D structures of ERα in complex with agonists and antagonists has given a molecular mechanism for agonism and antagonism, respectively.[28–30] When the ER LBD is complexed with estradiol or diethylstilbestrol (DES), H12 is positioned over the ligand-binding pocket, generating the AF–2 surface that promotes interaction with coactivators[29] and transcriptional activation. In contrast, in the ERα or ERβ LBD-raloxifene complexes[28,30] or in the ERα LBD–4-hydroxytamoxifen complex,[29] H12 was instead positioned in the hydrophobic cleft formed by H3, H4, and H5, foiling the coactivator interaction surface. (See also ch. 6, sec. 3.2.4 for molecular mechanisms of antiestrogen action.) It is evident that different ligands induce different receptor conformations[31,32] and that different conformations of the receptor affect the efficiency by which coactivators and corepressors interact with the liganded receptor[29] and, consequently, the agonist-antagonist profile of ligands.[33]

In recent years, there has been a strong focus on the cloning and characterization of nuclear receptor coactivators, corepressors, and their associated histone acetyl transferases (HATs) or deacetylases, respectively.[20] Integrator molecules like CREB (cAMP responsive element binding protein) binding protein (CBP/p300) and coactivator and corepressor proteins form protein:protein complexes with liganded nuclear receptors, bringing HATs or deacetylases in juxtaposition to chromatin. These events play a key role in the transcriptional regulation of target genes by liganded nuclear receptors and determine the final outcome on target gene expression.

The presence of different ER subtypes and isoforms and different coactivators and corepressors and our increasing knowledge of mechanisms by which ERα and ERβ, respectively, can modulate target gene expression have significantly added to our understanding of the physiology and pharmacology of estrogens and antiestrogens and have given a plausible explanation of why antiestrogens and SERMs sometimes behave more like estrogen agonists than estrogen antagonists. Initially it was believed that the ER affected the transcription of estrogen-sensitive genes only by direct binding of the ligand-activated receptor to EREs on DNA. We now know that ERα and ERβ also can modulate the expression of genes in an indirect manner, either by blocking the ability of a transcription factor to bind to its response element on DNA,[34] thereby inhibiting gene expression, or by stimulating gene expression by indirect binding of ER to DNA response elements through protein:protein interaction with other transcripton factors.[35–38] The different mechanisms by which ERα and ERβ modulate gene expression have changed our view of the pharmacology of estrogens and antiestrogens. The terms “agonism” and “antagonism” should be used with care. Natural or synthetic hormones that we now categorize as estrogen agonists and estrogen antagonists should perhaps not, in general terms, be categorized in this way. The reason for the caution is exemplified by the transcriptional effect of ERα and ERβ via an activator protein 1 (AP1) site in the presence of estrogens and antiestrogens.[39] With ERα, typical agonists such as estradiol and DES but also the antagonist tamoxifen function as equally efficacious agonists in the AP1 pathway, the antagonist raloxifene being only

The different mechanisms by which ERα and ERβ modulate gene expression have changed our view of the pharmacology of estrogens and antiestrogens.

a partial agonist. In contrast, with ERβ, estradiol acts as an antagonist inhibiting the agonistic activity of the two antagonists tamoxifen and raloxifene.[39]

In addition to the classical activation of the ERs by natural or synthetic hormones, alternative, indirect activation pathways of the ER (at least α) in the absence of ligand has been described[40–43] as a consequence of the activation of membrane receptors like those for insulin-like growth factor (IGF)-I,[44] epidermal growth factor (EGF)[45] and TGF and dopamine.[46] Still debated is the exact mechanism involved in this process. Several studies utilizing specific inhibitors of trandsduction signals, like ras, and protein kinase A and C (PKA and PKC), have clearly shown that the full activity of these molecules is essential for unliganded ER activation. Furthermore, point mutation studies have shown that two serines located in the N-terminal A/B domain are required for this process.[42] These findings indicate that phosphorylation of ER or of molecules interacting with ER is involved in transcriptional activation of the unliganded ER. More studies are necessary, particularly to better define whether this mechanism is conserved among different cell types. In fact, studies in various cell systems favor the hypothesis of differential mechanisms depending on the system examined.[47,48] The current hypothesis for the physiological significance of these alternative pathways implies that they may be of relevance in those phases of embryologic development in which neither estradiol nor its metabolites are available (e.g., during the maturation of the reproductive and the nervous systems).[47] These mechanisms might also be of pharmacological interest in the treatment of neoplastic forms that express the ER but have lost the responsiveness to treatment with ER antagonists (e.g., certain type of mammary carcinomas).

Another emerging and potentially important pathway involves the very rapid so-called nongenomic effects of ligands on nuclear receptors.[49] In endothelial cells, estrogen-ER complex-mediated membrane effects lead to sequential activation of ras, raf, mitogen-activated protein kinase kinase (MEK) and, subsequently, activation of mitogen-activated protein kinase (MAPK).[50] It is proposed that this may lead to activation of endothelial nitric oxide synthase (eNOS) and stimulated release of nitric oxide (NO). In neurons, membrane effects of estrogen lead to stimulation of src, ras, MEK, and MAPK, resulting in neuroprotection, and in the bone-specific osteoblasts, the membrane effects of estrogen may be involved in control of apoptosis, cell proliferation, and differentiation.[50]

An important aspect of the physiology and pharmacology of estrogens, which does not require the presence of the receptor protein, is their described antioxidant effects, suggested to protect from neurodegenerative disorders caused by oxidative stress, as in Alzheimer’s disease or atherogenesis due to excess uptake of oxidized low density lipoproteins (LDLs) by macrophages in the vascular wall.[51,52] Components of CEEs have also been tested for their antioxidant effects.[51] Equilin and its derivatives were reported to be better antioxidants than estradiol in inhibiting peroxidation of fatty acids and cholesterol in LDL particles. Other agents reported to exert neuroprotective or antiatherogenic effects caused by oxidative stress are phenolic compounds, vitamin E, insulin-like growth factor-1, and mifepristone (RU486).[51–55] In the context of antioxidant effects, antiestrogens (trans-hydroxytamoxifen, tamoxifen, and Imperial Chemical Industries PLC [(ICI) 182,780] but not E2 have been shown to activate the transcription of the quinone reductase gene and to increase NAD(P)H:quinone oxidoreductase enzyme activity via an electrophilic/antioxidant response element (EpRE/ARE).[56] Furthermore, E2 inhibited the agonistic effect of the antiestrogens, and ERβ was more efficacious than ERα in stimulating EpRE/ARE-containing reporter gene expression.[35] These findings suggest that antiestrogens are also potent antioxidants and stimulators of phase 2 detoxification enzyme genes, protecting cells from damage by radicals and other toxic byproducts of metabolic oxidation.

ERα gene polymorphisms may also provide important information about the physiology of estrogen action. Different ERα polymorphic forms have been linked to increased pig litter size[57] and human breast cancer susceptibility,[58] low BMD, osteoporosis,[59] hypertension,[60] spontaneous abortion,[61] and increased body height.[62]

2. Breast Tissue

The importance of estrogens in the development of female breast tissue is well documented. Female aromatase-deficient patients, unable to convert C^{19} steroids (e.g., testosterone) to estrogens, showed no sign of breast development at the onset of puberty[63]. Administration of estrogen to the two described female patients led to normal prepubertal and postpubertal breast development.

ERα knockout (ERKO or ERα-/-) female mice have lost their capacity to develop mammary gland tissue beyond the embryonic and fetal stages despite elevated levels of circulating estrogens (17β-estradiol). This impairment of breast development has been attributed to the lack of both direct and indirect (regulation of growth factor, e.g., EGF, and progesterone receptor (PR) expression) stimulatory effects of estradiol on breast epithelial and stromal tissues, due to missing ERα expression.[64] Applying tissue recombinant experiments and making a series of wild-type and ERα-/- breast stromal and epithelial tissue combinations, it was concluded that ERα expression in the stromal cell layer was essential for growth stimulation of the ductal epithelium in the mammary gland in mice.[65] PRL, also expressed and secreted from the anterior pituitary, plays a crucial role in mammary gland physiology. In ERKO female mice, the expression of PRL from the anterior pituitary and the circulating levels of PRL in serum are decreased by twentyfold and fivefold, respectively. It is conceivable that the impaired mammary tissue development in ERKO female mice is also due to decreased levels of PRL, in part due to lack of ERα expression in ERKO pituitary.[57]

More than 70 percent of primary breast cancers in women are ER (should be read ERα) positive and show estrogen-dependent growth that undergoes regression when deprived of supporting hormones. Patients whose breast tumors lack significant amounts of ERα rarely respond to endocrine ablation or treatment with antiestrogens, whereas most patients with ER-containing breast cancers benefit from such treatment.

The importance of estrogens in the development of female breast tissue is well documented.

Immunochemical determination of ER in tumor biopsies has become a routine clinical procedure on which the choice of therapy is based. However, the currently available immunochemical procedures for ER measurements are based on ERα-specific antibodies that do not detect ERβ protein (unpublished observations).

ERβ mRNA and protein, together with ERα mRNA and protein, have been detected in human breast cancer biopsies and in human breast cancer cell lines.[66] With the use of receptor-specific antibodies, both ERα and ERβ were found to be expressed in the normal rat mammary gland, but the presence and cellular distribution of the two receptors were distinct.[67] While the level and percentage of cells expressing ERβ was more or less constant during prepubertal and pubertal stages and throughout pregnancy, lactation, and postlactation, the level and percentage of ERα-containing cells varied dramatically. The possible role of ERβ in normal breast tissue development and physiology and in breast cancer development and/or therapy is, however, as yet unknown.[68]

3. Urogenital Tract

ERα and ERβ are both expressed in uterus, ovary, testes, and prostate, but with different cellular localization. In the ovary, ERα is mainly expressed in thecal cells and in the prostate mainly in the stromal compartment. ERβ is expressed mainly in glandular epithelium of the uterus, primarily in the granulosa cells of the ovary, and mainly in the epithelium of the testes and prostate.

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Aromatase-deficiency in female patients led to excess circulating androgens in the fetus and at puberty, resulting in virilization and ambisexual development. The two aromatase-deficient female patients[63] were reported with ambiguous genitalia at birth, a phenotype that was further pronounced at pubertal age, and with polycystic ovaries, characterized by a disproportionate number of atretic follicles and dense fibrotic subcortical stroma. The elevated serum levels of FSH and LH in these patients, as a result of perturbed estrogen-dependent negative feedback on gonadotropin production, were suspected to be the cause of the polycystic ovaries. Estrogen replacement in these affected female patients led to normalized gonadotropin and androgen levels, resolution of the ovarian cysts, and menarche.[63] Like female aromatase-deficient patients, aromatase knockout (ArKO) female mice had low serum estrogen levels and high testosterone and gonadotropin levels.[69] Female ArKO mice also displayed genital anomalies, with underdeveloped external genitalia and uteri; and the ovaries contained numerous follicles that appeared arrested before ovulation. The stroma of the ovaries was hyperplastic, with structures that appeared to be atretic follicles. No corpora lutea were present.[69]

Male patients with either defective estrogen production[63] and the male patient with estrogen insensitivity caused by a nonsense mutation in ERα gene[70] are reported to have macroorchidism or oligozoospermia and/or decreased sperm viability or motility. The fertility of the aromatase-deficient or estrogen-resistant male patients is not known. Male ArKO mice were initially fertile but developed progressive infertility[71] due to arrested spermatogenesis. These findings suggest that estrogen has a direct effect on male germ cell development and fertility.

Deletion of the ERα gene in mice results in infertility in both females and males. ERα-/- female mice show complete infertility with hypoplastic, estrogen-resistant uteri and hyperemic ovaries with no ovulatory capacity.[64] Similar to the aromatase-deficient female patients, ERKO female mice have elevated testosterone and LH levels. Treatment of these mice with a GnRH antagonist reduced the serum levels of LH and reverted or prevented the cystic ovarian phenotype,[72] in agreement with the disappearance of polycystic ovaries in aromatase-deficient female patients following estrogen substitution and subsequent normalization of serum gonadotropin levels. To challenge the ovulatory deficiency of ERKO female mice, immature mice were treated with exogenous gonadotropins. Although the ovulatory capacity was reduced compared with age-matched wild-type mice, the collected oocytes were fully competent to undergo successful in vitro fertilization,[72] suggesting that ERα is not critical for follicle maturation and ovulation.

ERβ knockout (BERKO or ERβ-/-) mice also have been generated. Female animals showed reproductive defects (20 percent of normal fertility),[64] while males showed normal fertility. The LH and estrogen levels in BERKO females are comparable to wild-type, but their fertility is compromised due to reduced ovarian efficiency. Superovulation of BERKO female mice exhibited several mature but unruptured follicles. The number of corpora lutea was considerably less than in wild-type mice, suggesting an attenuated response to the ovulatory hormone surge in the absence of ERβ.

Female mice unable to express either ERα or ERβ, DERKO mice, exhibited normal reproductive tract organ development but were, as expected, infertile.[16] Similar to ERKO female mice, the DERKO females showed uterine hypoplasia but no polycystic ovaries. The ovaries of prepubertal DERKO females, displayed precocious maturation evidenced by multiple, large antral follicles, not observed in control wild-type females. The very high serum levels of LH in these animals explain the prepubertal, precocious ovary phenotype of the DERKOs.[16] The ovarian phenotype of the adult DERKO female was distinct from the ERKO and BERKO female phenotypes, most notably by the presence of structures resembling seminiferous tubules of the testis. The sex-reversal of the adult DERKO ovary phenotype was judged to be caused by a redifferentiation of ovarian components rather than by a developmental phenomenon.[16] In summary, based on the ovarian phenotype in DERKO females, it was concluded that both ERα and ERβ are required for the maintenance of germ and somatic cells in the postnatal mouse ovary.

Male ERKO mice are infertile, with atrophy of the testes and seminiferous tubule dysmorphogenesis resulting in decreased spermatogenesis and inactive sperm.[64] Recently, a more detailed study of the cause of the infertility of male ERKO mice provided biological evidence that ERα plays an important role in the reabsorption of luminal fluid from the efferent ductules during the transit of spermatozoa from the testis to the head of the epididymis.[64] Concentration of sperm is claimed to improve their survival and maturation during epididymal storage. Administration of high levels of estrogen to men is known to cause infertility. Thus, another possible explanation that may contribute to the nonreproductive phenotype of ERKO male mice is the relatively high levels of estrogens in ERKO mice and the presence of ERβ in seminiferous epithelium, spermatids, and spermatocytes, causing infertility by a direct action on the testes. In contrast to male ERKO mice, male BERKO mice are fertile,[64] suggesting a different role for ERβ compared to ERα in the male reproductive system. As expected, DERKO male mice are infertile, with an 80-percent reduction in the number of sperm produced in the testis.[16]

Estrogens are claimed to be effective in the treatment of urge incontinence in postmenopausal women. It has recently been shown that ERβ is highly expressed in the inner epithelial cell layer of the rat bladder and urethra.[73] These results suggest that female patients with UI might benefit from ERβ-selective agonist therapy.

Estrogens have also been linked to prostate disease. In different species, estrogens synergize with androgens in inducing glandular hyperplasia and dysplasia and in inducing adenocarcinoma in the prostate.[74] Immunohistochemical studies revealed that ERβ is the predominant ER subtype in the prostate, located in the epithelial cells along the ductal network of the prostate. ERα has been detected only in the stromal compartment of the prostate.[73–75] It has been suggested that ERβ is regulated by androgens in the prostate, since the abundance of ERβ mRNA was rapidly reduced following castration but restored after testosterone replacement.[76] Exposure of wild-type mice to the estrogen 5α-androstane-3β,17β-diol, a metabolite of dihydrotestosterone, caused a decrease in the level of androgen receptor (AR) in the prostate.[75] In ERβ-/- mice, however, the level of AR is elevated, and 5α-androstane-3β, 17β-diol was without effect, suggesting that the AR gene is an ERβ target in the prostate.[75] Exogenous estrogens have a negative effect on epithelial cell differentiation, ductal morphogenesis, and prostate growth,[74] and the prostate of adult rats neonatally exposed to estrogens shows hyperplasia, dysplasia, and presence of *in situ* carcinoma. It was hypothesized that ERβ is a marker of epithelial differentiation and that the decline in epithelial cells in neonatally estrogenized rats is a result of altered epithelial cell differentiation.[74,76] ERβ-/- mice display signs of prostatic hyperplasia with aging.[75] This suggests that ERβ may protect against abnormal prostate growth.

4. Bone: Development and Homeostasis

It is well established that estrogens exert an important influence on bone physiology; clinically this is manifested by the occurrence of osteoporosis in postmenopausal women. There is also compelling evidence that estrogens protect postmenopausal women from bone loss and the development of osteoporosis, maintaining a balance between bone resorption and bone formation.[77,78] The level of estrogens may play a more important role than testosterone for the maintenance of bone mass also in aging men,[79,80] with a positive correlation between BMD and serum estradiol concentrations rather than testosterone levels.

As in other tissues, there are most likely both direct and indirect effects of estrogens in maintaining a balanced bone metabolism. The likelihood of important direct effects of estrogens on bone is based on the presence of ERα in the bone-forming osteoblasts[81,82] and in the bone-resorbing osteoclasts.[83] ERβ mRNA has been found in primary rat osteoblasts, in rat osteosarcoma cells,[84] and in immortalized human fetal osteoblasts.[85] Evidence for indirect effects of estrogens on bone metabolism stems from studies in mice, rats, and humans,[86–91] suggesting a coupling and cooperativity between GH and estrogen in bone metabolism. Taken together, these studies indicate that estrogen substitution can increase the circulating levels of GH[87] and the levels of GH receptor on osteoblasts[90] and that there is a mutual dependence of GH and estrogen action on bone growth, mineral density, and maintenance.[86,88,89,91] In addition, estrogens may have indirect effects on osteoclast differentiation, maturation, and activity by inhibition of cytokine expression[92–95] and via stimulation of osteoprotegerin expression from human osteoblasts.[96] The importance of these interactions for the maintenance of bone health needs to be evaluated.

Despite approximately tenfold higher levels of circulating estradiol in ERα-/- mice, there was a significant decrease in the length and size of the femur in females but only slight decrease in males.[64] In contrast, the decrease in BMD and BMC was more pronounced in ERKO males than females.[64] In BERKO mice, the bone phenotype of male mice was unaffected compared to wild-type male mice, while there was a masculinization of the long bones (femur) in the female BERKO mice.[97] Lack of ERβ expression in the female BERKO mice led to increased length of the femur, thicker cortical bone (increased BMC due to increased periosteal circumference), and increased size of the vertebrae, approaching the corresponding characteristics of wild-type male mice. There was no effect on trabecular architecture or BMD in the male or female BERKO mice. Ovariectomy of female mice leads to loss in trabecular BMD to a similar extent in both BERKO and wild-type animals,[97] suggesting an important role for ERα in the maintenance of trabecular BMD and architecture in mice. A further support for the importance of ERα in bone physiology was obtained from examination of a male estrogen-insensitive patient with a nonsense mutation of the ERα gene.[70] Similar to the ERKO mice, he had elevated levels of LH, FSH, and estrogen. Despite the elevated levels of estrogen, he had low BMD and continuous linear growth because of unfused epiphyses, suggesting an important role for ERα in human bone biology. The decrease in length and size of femur in the female ERKO mice may be indicative of an effect of ERβ in the presence of excessive amounts of estradiol or its metabolites. The possible effects of disrupted ERα and ERβ expression in ERKO and BERKO mice, respectively, on GH expression and its consequences for the bone phenotype in these animals are not yet known.

Male and female patients with aromatase-deficiency[63] have increased bone turnover, delayed bone maturation, low BMD, and tall stature due to unfused epiphyses. They have elevated circulating levels of androgens, FSH, and LH but very low or undetectable levels of estrogen. ERT of both female and male aromatase-deficient patients resulted in growth spurt, closure of the epiphyses, and increased BMD,[63] suggesting a very important role for estrogens not only in females but also in males.

The pubertal growth spurt starts earlier in girls than in boys,[98] beginning at midpubertal stage in boys. The average duration of pubertal growth spurt in girls is shorter than in boys, possibly explained by higher levels of estrogen in prepubertal girls than in prepubertal boys, hypothesized to cause a more rapid skeletal maturation and epiphyseal closure in girls than in boys. Using an ultrasensitive assay for determination of serum estrogen levels, the rise and decline in estrogen levels in boys have been assessed in correlation to age, pubertal growth peak velocity, bone maturity, and epiphyseal closure.[99] In this study, there was a close correlation between the rise in estrogen level and the rise in the level of testosterone, and the rise in estrogen level correlated with the time of peak growth velocity.[99] Following growth spurt in these boys, there was a further increase in estrogen levels that was sustained toward the end of puberty, hypothesized to accelerate epiphyseal fusion.[99]

5. Cardiovascular System

Women's risk for the occurrence of CVD clinical events at an early age is less than that for men. The CVD risk increases with age for women, approaching the same incidence rate as for men, starting from the age of 50. (See also ch. 8.) Based on observational epidemiological studies, HRT is described to have a cardiovascular protective effect in postmenopausal women, decreasing the risk of developing atherosclerosis and CVD. (See ch. 8.)[100–102] The cardioprotective effect of estrogens is debatable as no such data from clinical trials are available yet. On the other hand, the outcome of HERS,[103] which did not show any overall cardiovascular benefit in postmenopausal women (treated with oral CEE plus MPA) with established CHD, may be explained on the basis of recent findings, according to which medroxyprogesterone antagonizes positive effects of estrogens on the vasculature. (See ch. 8, sec. 3.2.) The recommendation drawn from HERS was not to initiate hormone treatment for secondary prevention of CHD. Despite an early increase in risk in treated women, fewer CHD events were observed over time in the hormone treatment compared with the placebo group; it was therefore recommended that women with CHD already on HRT may well continue the treatment.

ERα and ERβ are expressed in vascular endothelial cells,[102] smooth muscle cells,[104] and myocardial cells.[105] A number of direct effects of estrogen on vascular tissue have been reported:[101,102,106,107] nongenomic vasodilation as an effect of estrogen on ion-channel function[108] and NO synthesis,[109] long-term effects by modulation of prostaglandin synthase, NO synthase and endothelin gene expression,[105,110] regulation of angiotensin AT1 receptor density on vascular smooth muscle cells,[111] and inhibition of injury induced vascular intimal thickening.[112] Furthermore, reduced heart contractility in ovariectomized female rats normalized following estrogen replacement,[113] an effect, in part, explained by estrogen-mediated changes in expression of contractile proteins.[106,114]

Besides a higher risk for men to develop atherosclerosis and CVD at an early age compared to women, there are other gender-related cardiovascular differences.

Besides a higher risk for men to develop atherosclerosis and CVD at an early age compared to women, there are other gender-related cardiovascular differences. Men have a higher prevalence of left ventricular hypertrophy than women,[106,115,116] hypothesized as an effect of the difference in the level of circulating estrogens in men compared to women[106] but possibly also due to gender-specific differences in ER levels and in the induction of endogenous gene expression in cardiac myocytes in response to estrogen.[106,105] In rats, intimal thickening after vascular injury is significantly greater in males than in females.[107] Male level of intimal thickening occurred in female rats after ovariectomy, an effect that was reversed by estrogen therapy.[112] The primary inhibitory effect of estrogen on intimal thickening was mediated by its direct effect on vascular smooth muscle cells, inhibiting their migration and proliferation.[112] Another gender difference is the rapid response to estrogen after acetylcholine-induced coronary arterial constriction in women and men with coronary artery disease (CAD).[117] In female patients, administration of estrogen reversed the vasoconstriction response to acetylcholine, while there was no response to estrogen in male patients. Coronary blood flow was significantly enhanced in the presence of estrogen in the female patients, but no response to estrogen occurred in the men. A plausible explanation for these differences may be that vascular endothelium in women produces more NO in response to estrogen than in men. The specific role of ERα and ERβ in maintaining normal cardiovascular function and in prevention of the development of atherosclerosis and CVD is still largely unknown.

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However, disruption of the ERα gene, as in ERKO mice, showed a reduced production of NO.[64] ERα also seems involved in neovascularization, as there was no angiogenic response to estrogen in ERKO mice.[64] An increased number of L-type Ca^{2+} channels was reported in ERKO male mice,[64] suggesting an involvement of estrogen and ERα in the regulation of cardiac excitability. In the man bearing an ERα nonsense mutation,[64] there was absence of endothelium-dependent vasodilation of the carotid arteries following ischemic cuff occlusion.[118] However, this lack of ischemic response may relate more to atherosclerosis-induced endothelial dysfunction than to a direct consequence of lack of ERα-mediated responses in the vascular endothelium.[107,119] Sublingual estrogen delivery caused a rapid vasodilator response in the ERα-deficient man,[118] possibly suggesting a role for ERβ in rapid vascular responses to estrogen. In contrast, there was no negative effect of vascular endothelium-dependent vasodilation in ERα-/- mice.[64] Estrogen treatment protects against vascular injury, suppressing smooth muscle cell proliferation and intimal thickening, in ERKO mice.[64] The expression of ERβ but not ERα was dramatically increased in wild-type mice after vascular injury.[102] These data suggest an important role for ERβ in vascular injury response and protection. However, a similar estrogen-dependent vascular injury protection was also seen in BERKO mice, suggesting an ERα and ERβ redundancy in vascular injury protection or that an unknown signaling pathway or a still unidentified ER is involved.[102] In this context, the estrogen resistant man[70] showed intimal thickening of the common carotid arteries[118] despite elevated circulating levels of estrogen.

Part of the beneficial effects of estrogen on cardiovascular function and reactivity relates to liver-specific effects, regulating serum lipid-cholesterol, levels.[101] Estrogens increase the level of apolipoprotein A1 in postmenopausal women.[120] Apolipoprotein(a), the major protein component of the atherogenic lipoprotein (Lp(a)), is down-regulated in the liver by estrogen at the mRNA level resulting in decreased plasma levels of Lp(a).[121] Estrogen also increases the expression of angiotensin in postmenopausal women,[122] regulates the level of HMG CoA reductase at the protein level, and increases LDL receptors on the surface of liver cells.[101] Regulation of the apoE gene by estrogen has been demonstrated in the rat[123] and mouse.[64]

As in skeletal tissue, GH may play an important role for estrogen effects in liver,[91] with hypophysectomy blunting the cholesterol-lowering effect of estrogen in ovariectomized rats.[91] The liver in female rats has more GH receptors than in male rats,[124] and liver GH receptor expression positively correlates to estrogen status in female and male rats.[125]

So far ERα but not ERβ has been shown to be expressed in liver;[126] thus all effects of estrogen reported on liver-specific gene expression are ERα mediated. Further support for the physiological role of ERα and estrogens in the regulation of liver-specific gene expression and lipid-lipoprotein homeostasis stems from the analysis of the ERα-deficient[70] and the aromatase-deficient patients[63] and from the ERKO mice,[64] demonstrating glucose intolerance and lipid abnormalities as a consequence of estrogen resistance or estrogen insufficiency.

In addition to liver and cardiovascular cells, monocytes-macrophages also are involved in health and disease of the cardiovascular system.[127,128] Several studies, both *in vitro* and *in vivo*, have indicated that growth factors and cytokines that mediate the critical processes of inflammation and wound healing also play a central role in vascular disease and during the initiation and progression of atherosclerosis. The cytokines interleukin-1b (IL–1b) and tumor necrosis factor α (TNFα) have been implicated in the processes of vascular injury and atherogenesis.[129] Both ERα and ERβ are reported to be expressed in monocytes-macrophages.[130] Estradiol has been shown to inhibit LDL oxidation, to inhibit and cholesteryl ester formation and accumulation in macrophages,[131] and to reduce the uptake of acetylated LDL into macrophages, resulting in a reduced rate of foam cell formation.[132] Estrogen down-regulates the expression of TNFα in human macrophages[133] by a mechanism that involves ERβ but not ERα.[134] These results suggest that monocytes-macrophages also are potential targets for the protective effects of estradiol on the cardiovascular system and the development of atherosclerosis.[128]

6. Central Nervous System and the Hypothalamo-Pituitary Axis

Estrogens are reported to influence a variety of functions in the CNS, such as learning, memory, awareness, fine motor skills, temperature regulation, mood, and reproductive functions.[135] Estrogens are also linked to symptoms of depression and treatment of depressive illness. (See also ch. 12.)

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Different brain structures and neurotransmitter systems are involved in the different effects of estrogens.[135] The serotonin 5–hydroxytryptamine (5–HT) system, with neurons projecting from the dorsal and medial raphe of the midbrain/brainstem raphe nuclei to multiple forebrain areas such as the hypothalamus, hippocampus, and cortex,[135] is involved in the modulation of reproduction, mood, sleep, and cognition. Serotonin levels and activity in CNS are altered by serum estrogen fluctuation in rodents, and estrogen substitution in ovariectomized rats positively affects the serotonergic system.[135,136] Estrogen has been reported to increase the expression of tryptophan hydroxylase (TPH), the rate-limiting enzyme for serotonin synthesis,[137] and to suppress the expression of the serotonin reuptake transporter (SERT) in raphe nuclei of ovariectomized monkeys.[138] Estrogen reduced the level of the 5–HT_{1A} autoreceptor subtype in the dorsal raphe nucleus of spayed monkeys[139] and reduced agonist stimulated 5–HT_{1A} receptor inhibition of dorsal raphe neuron firing in rats,[140] suggesting that estrogen may facilitate 5–HT neurotransmission.[139] The level of postsynaptic 5–HT_{1A} receptors, mainly localized in the limbic brain areas and the cerebral cortex, is also affected by estrogen.[141] 5–HT_{2A} receptors, suggested to be involved in the control of hormone and transmitter release, control of sexual activity, regulation of sleep, motor behavior, and psychiatric disorders such as anxiety and depression,[142] are positively regulated by estrogen in the dorsal raphe, olfactory bulb, and cerebral cortex.[143–145] In male rats, 5–HT_{1A} receptor levels also are regulated by estrogen.[146] ERβ mRNA has been reported within the dorsal raphe of the rat,[147] and an ERα immunoreactive protein has been detected in neurons adjacent to serotonergic cells in rat dorsal raphe.[135] An autoradiographic study in ERα-/- mice indicated an abundant presence of ERβ in mouse dorsal raphe,[148] and no ERα protein has been detected in the macaque raphe,[135] suggesting that ERβ may play a more important role than ERα in mediating estrogen effects on the serotonergic system.

The dopaminergic system, involved in motor function, motivation, reward, cognition, and hypothalamic-pituitary control, is also affected by estrogen.[149,150] Dopamine levels and turnover fluctuate during the estrous cycle,[151] and administration of estrogen, following ovariectomy, potentiates the release of dopamine.[152,153] Estrogen also increases dopamine transporter binding sites in the striatum[154] as well as the densities of dopamine receptors D_1 and D_2.[155] In the hypothalamus, the dopaminergic tuberoinfundibular neurons inhibit PRL release from the anterior pituitary by release of dopamine into the hypophyseal portal system, an effect that is inhibited by estrogen.[156]

The basal forebrain cholinergic neurons project to the cerebral cortex and hippocampus and are implicated in learning and memory.[135] Long-term ovariectomy results in impaired learning due to decline in high-affinity choline uptake and choline acetyl transferase (ChAT) activity in rats.[135] Estrogen substitution following ovariectomy in rats induced ChAT enzyme levels and increased ChAT activity in the basal forebrain and possibly ChAT activity in projection areas ending in the cerebral cortex and hippocampus.[135] ChAT mRNA levels fluctuate in the basal forebrain cholinergic neurons during the estrous cycle in the rat.[135] The colocalization of ERα with nerve growth factor (NGF) receptors in cholinergic neurons of the rat basal forebrain,[135] and the stimulation of estrogen of both NGF receptor mRNA and ChAT mRNA in the rat basal forebrain,[135] suggest a possible role for ERα in learning and memory functions. However, the predominant expression and localization of ERβ in rat neocortex, hippocampus, and nuclei of the basal forebrain suggest an important role for ERβ in learning and memory.[147,157] This assumption is further supported by the maintained normal memory and learning function in ERKO mice.[64] In a recent study on human brain, the predominant presence of ERβ message in the hippocampal formation, entorhinal cortex, and thalamus suggests a putative role of ERβ in cognition, memory, and motor functions.[158]

Additional transmitter systems shown to be influenced by estrogens are vasopressin and oxytocin,[159,160] somatostatin,[161] galanin,[162] the γ-aminobutyric acid (GABA) system,[163] and the glutamate system.[164,165]

The expression patterns of ERα and ERβ, based on mRNA, autoradiographic, or immunohistochemical studies of rat and mouse brain, indicate a more abundant or distinct presence of the two ER subtypes in certain areas of the brain but also areas where they seem to overlap. ERα seems to be more abundant in the hypothalamus (preoptic, arcuate, periventricular, and ventromedial nuclei) and amygdala (amygdala hippocampal area, medial and cortical nuclei).[147,166] High levels of ERβ mRNA have been found in the medial preoptic, paraventricular and supraoptic nuclei of the rat hypothalamus. In the amygdala, ERβ is primarily expressed in the medial amygdala nucleus. Moderate to high ERβ mRNA levels are found in olfactory bulbs, bed nucleus of the stria terminalis, hippocampus, cerebral cortex, cerebellum, midbrain raphe, and basal forebrain.[135,147,148,157,166–168]

The HPA regulates overall endocrine homeostasis in the body. Estrogen, through effects on the HPA, modulates the expression and secretion of several hormones from the anterior pituitary gland, such as LH, FSH, GH, and PRL.[64] Both ERα and ERβ are expressed in the pituitary gland, but ERα predominates,[64] in particular in the gonadotrophs and lactotrophs. Both ER subtypes are also expressed in the preoptic area of the hypothalamus and are believed to be involved in regulating the expression of pituitary hormones, but ERβ predominates.[147]

Although serum levels of LH and FSH are directly controlled by hypothalamic GnRH, it is the circulating level of estrogen, other sex steroids, and the inhibin glycoproteins that are the most important physiological determinants of serum gonadotropin levels.[64,169,170] There is a strong inverse correlation between the circulating levels of inhibin and FSH. The main source of inhibin (inhibin A and inhibin B) production in females is the ovary, inhibin B being expressed in the early follicular phase with a peak at the mid-follicular phase and inhibin A being expressed by the dominant follicle and the corpus luteum with a peak in the late follicular phase and in the midluteal phase.[169,170] In men, inhibin B, proposed to be the main inhibin involved in FSH regulation, is primarily produced by the Sertoli cell.[169]

Female and male patients with aromatase-deficiency have elevated levels of LH and FSH, elevated circulating levels of androgens, but very low circulating levels of estradiol and estrone.[63] Therapy with conjugated estrogens in both female and male aromatase-deficient patients resulted in normalization of gonadotropin and testosterone levels.[63] Clinical data on the male patient with the ERα nonsense mutation[70] also showed increased serum LH and FSH levels despite normal levels of testosterone and high estrogen levels. Transdermal ethinyl-estradiol therapy of this man did not lower serum LH or FSH. Estradiol substitution of ovariectomized rats prevented the expected increase in LH but only partially blocked the rise in FSH. In ERα-/- mice, the circulating LH levels, but not FSH, are elevated despite tenfold higher serum levels of estrogen.[64] Taken together, these data indicate that estrogen is more important than testosterone (also in men[171–173]) in regulating circulating gonadotropin levels and that ERα plays a major role in mediating the effect of estrogen in this process. The effect of activin-inhibin feedback regulation of pituitary FSH expression is independent of ERα. Whether ERβ has a role in ovarian activin-inhibin expression and the feedback regulation of gonadotropin expression remains to be investigated.

LH and FSH surge is critical to female ovarian cycle and fertility;[64] elevated estradiol levels in proestrous are required for the preovulatory LH surge from the anterior pitutary, triggered by a discharge of GnRH into the hypophyseal portal system.[174] The anteroventral periventricular nucleus (AVP) of the preoptic region, a sexually dimorphic part of the hypothalamus, is thought to play a critical role in transducing the gonadotropin surge.[64] The AVP is larger in female mice and contains a greater number of dopaminergic neurons than in males.[64] Testosterone exposure of neonatal females reduces the number of dopaminergic neurons and precludes an LH surge. The AVP provides direct projections to a subpopulation of GnRH neurons in the preoptic region that are thought to participate in the initiation of the preovulatory LH surge.[64] Also progesterone and the PR are necessary components of the LH surge.[64,175] Both ERα and ERβ have been shown to trigger PR expression in the preoptic nucleus,[167] suggesting that either of the two ER subtypes or both may participate in triggering the LH surge. Also other neurotransmitter systems in the brain are suggested to contribute to the induction of the LH surge.[64] ERα-containing histaminergic neurons located in the tuberomammillary complex were shown to be involved in the positive feedback effect of estrogen in the induction of the LH surge, mechanistically via histaminergic axo-dendritic and axo-somatic appositions onto GnRH neurons and the histamine H1 receptor.[174]

7. Hormone Replacement Therapy: Traditional Alternatives and Future Perspectives

The most common regimens used to treat symptoms of the menopause and postmenopausal health risks, such as osteoporosis and CVD, are 17β-estradiol, esterified estrogens, or CEEs, each in combination with a progestin.

The most common regimens used to treat symptoms of the menopause and postmenopausal health risks, such as osteoporosis and CVD, are 17β-estradiol, esterified estrogens, or CEEs, each in combination with a progestin, for example, MPA, to avoid increased risk of endometrial or uterine cancer in women with an intact uterus. Another combination used is estrogen with testosterone, claimed to increase libido and decrease depression. The awareness of undesired effects (e.g., resumption of monthly bleeding, breast tenderness, and headaches) or health risks (breast cancer, endometrial cancer, venous thromboembolism, ovarian cancer, asthma, and gall bladder disease) with existing HRT (first generation HRT) warrants alternatives with improved safety profiles. Recently developed nonsteroidal ER ligands with mixed agonist-antagonist activity, the so-called SERMs (second generation HRT), display a tissue-selective estrogen agonism in for example, bone and liver, but estrogen antagonism in breast and uterine tissue.[176–178] Additional ER ligands with similar mixed agonist-antagonist activity (SERMs) are in development.[177] Although the increased risk of breast cancer and endometrial cancer is obviated by the SERMs, they have not shown the same efficacy as estrogen to prevent bone fractures of the hip, though they have proved efficacious in reducing vertebral fracture risk. Current SERMs increase the incidence of or aggravate hot flushes in postmenopausal women, and the incidence of venous thromboembolism is the same as for first generation HRT. A serious disadvantage for this category of drugs became evident as the SERMs levormeloxifene and idoxifene were withdrawn from further development due to increased incidence of UI and uterine prolapse in postmenopausal women. The discovery of a second ER subtype, ERβ, has revitalized the search for improved drugs for HRT that most likely will better provide the benefits of ERT. Several large pharmaceutical companies are engaged in the development of ERα- and ERβ-selective SERMs (third generation HRT), but as yet there is no information regarding their development. However, synthetic ER subtype-selective ligands have been reported.[179] The most ERα-selective ligand showed 120-fold higher agonist potency for ERα than for ERβ. Another ER subtype-selective ligand, synthesized by the same group, showed full ERα agonism but pure ERβ antagonism.

Current alternatives for women who do not wish to take the HRT cited above are: (1) synthetic progestins, megestrol acetate (a synthetic derivative of androgens), or tibolone (a synthetic steroid with estrogenic, progestational, and androgenic activity) for alleviation of hot flushes; (2) bisphosphonates or calcitonin for prevention of osteoporosis; and (3) statins or antioxidants for prevention of CVD.[176]

8. Future Needs

Further characterization of the phenotypes of ERα and ERβ knockout mice will be of continuing importance, not least regarding the effects of ERα and/or ERβ deficiency in aging mice. That ERα/ERβ double knockout mice are viable needs an explanation: the role of redundant systems to secure viability and functionality, the importance of membrane or non-genomic effects of estrogens, and/or the possible existence of a third ER subtype have to be clarified. Other compelling questions to be answered about the biological role of ERα and ERβ stem from the observation that both ERα and ERβ are expressed in normal and malignant breast tissue. Phenotypic characterization of the ERβ-/- mice revealed a role for ERβ as an antiproliferative receptor. In several tissues it operates to oppose the effects of ERα (yin-yang principle). Male BERKO mice develop prostate hyperplasia, which becomes malignant with age, and aging female BERKO mice develop lymphoma. Furthermore, BERKO mice have severely impaired ovarian function related to the dysregulation of AR. Treatment of BERKO females with antiandrogens reversed the phenotype. We have concluded that the major role of ERβ in the ovary is down-regulation of the AR in maturing follicles. In BERKO mice ovaries, AR remains high, for example, the ovary is in a hyperandrogenic state and is similar to ovaries seen in polycystic ovarian disease in humans. There is a need not only to identify genes, which are regulated by ERβ, but also to understand the regulation of the ERβ gene itself. Furthermore, it will be of importance to identify possible mutations in ERβ and to investigate the role mutated ERβ might play in human diseases.

Priority issues:

- Understand whether breast tumors arise in cells that already contain one or more of the ERs.
- Investigate the roles of the two ERs in the breast (synergistic or opposing).
- Compare BERKO mice susceptibility to development of breast cancer to that of controls or of ERKO mice.
- Determine whether both ERα- and ERβ-containing stromal cells secrete growth factors in response to estrogens.
- Investigate, if present, the role of mutations in ERβ in human breast cancers.
- Recognize the role of the splice variants of ERβ in the normal breast and in breast malignancy.
- Unravel the mechanism of activation of ER in postmenopausal breast.
- Identify genes that are regulated by ERα and ERβ in normal breast and in breast malignancy.

Current SERMs increase the incidence of or aggravate hot flushes in postmenopausal women, and the incidence of venous thromboembolism is the same as for first generation HRT.

More specifically there is a need to:

- Understand why epithelial cells in the prostates of ERβ knockout mice are never in G0 and identify the stage in the cell cycle in which they are arrested.
- Identify the genes involved in the change from hyperplasia to malignant phenotype in the prostate.
- Characterize the lymphoma which develops in BERKO females.
- Characterize the role of ERβ in the immune system.
- Investigate the role of ERβ mutations in women with polycystic ovarian syndrome.

References

[1] Jensen EV, Jacobsen HI. Basic guides to the mechanism of estrogen action. *Recent Prog Horm Res* 1962;18:387–414.

[2] Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. *Science* 1986;231(4742):1150–4.

[3] Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-Å. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996;93(12):5925–30.

[4] Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett* 1996;392:49–53.

[5] Enmark E, Pelto-Huikko M, Grandien K, et al. Human estrogen receptor beta - gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab* 1997;82(12):4258–65.

[6] Tremblay GB, Tremblay A, Copeland NG, et al. Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor beta. *Mol Endocrinol* 1997;11(3):353–65.

[7] Ogawa S, Inoue S, Watanabe T, et al. The complete primary structure of human estrogen receptor beta (hER beta) and its heterodimerization with ER alpha in vivo and in vitro. *Biochem Biophys Res Commun* 1998;243(1):122–6.

[8] Leygue E, Dotzlaw H, Lu B, Glor C, Watson PH, Murphy LC. Estrogen receptor beta: mine is longer than yours? *J Clin Endocrinol Metab* 1998;83(10):3754–5.

[9] Chu S, Fuller PJ. Identification of a splice variant of the rat estrogen receptor beta gene. *Mol Cell Endocrinol* 1997;132(1-2):195–9.

[10] Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG, Brown TA. Identification of estrogen receptor beta 2, a functional variant of estrogen receptor beta expressed in normal rat tissues. *Endocrinology* 1998;139(3):1082–92.

[11] Moore JT, McKee DD, Slentz-Kesler K, et al. Cloning and characterization of human estrogen receptor b isoforms. *Biochem Biophys Res Commun* 1998;247(1):75–8.

[12] Ogawa S, Inoue S, Watanabe T, et al. Molecular cloning and characterization of human estrogen receptor beta cx: a potential inhibitor of estrogen action in human. *Nucleic Acids Res* 1998;26(15):3505–12.

[13] Zhang QX, Hilsenbeck SG, Fuqua SA, Borg A. Multiple splicing variants of the estrogen receptor are present in individual human breast tumors. *J Steroid Biochem Mol Biol* 1996;59(3–4):251–60.

[14] Friend KE, Resnick EM, Ang LW, Shupnik MA. Specific modulation of estrogen receptor mRNA isoforms in rat pituitary throughout the estrous cycle and in response to steroid hormones. *Mol Cell Endocrinol* 1997;131(2):147–55.

[15] Karas RH, Hodgin JB, Kwoun M, et al. Estrogen inhibits the vascular injury response in estrogen receptor beta-deficient female mice. *Proc Natl Acad Sci USA* 1999;96(26):15133–6.

[16] Couse JF, Hewitt CS, Bunch DO, et al. Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science* 1999;286(5448):2328–31.

[17] Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* 1994;63:451–86.

[18] Giguère V. Orphan nuclear receptors: from gene to function. *Endocr Rev* 1999;20(5):689–725.

[19] Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. *Cell* 199;83(6): 835–9.

[20] Shibata H, Spencer TE, Onate SA, et al. Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Prog Horm Res* 1997;52:141–5.

[21] Berry M, Metzger D, Chambon P. Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO* J 1990;9(9):2811–8.

[22] McInerney EM, Tsai MJ, O'Malley BW, Katzenellenbogen BS. Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. *Proc Natl Acad Sci* USA 1996;93(19):10069–73.

[23] Webb P, Nguyen P, Shinsako J, et al. Estrogen receptor activation function 1 works by binding p160 coactivator proteins. *Mol Endocrinol* 1998;12(10):1605–18.

[24] Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J-Å, Nilsson S. Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. *Mol Pharmacol* 1998;54(1):105–12.

[25] McInerney EM, Katzenellenbogen BS. Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. *J Biol Chem* 1996;271(39):24172–8.

[26] McInerney EM, Weis KE, Sun J, Mosselman S, Katzenellenbogen BS. Transcription activation by the human estrogen receptor subtype beta (ER beta) studied with ER beta and ER alpha receptor chimeras. *Endocrinology* 1998;139(11):4513–22.

[27] Mak HY, Hoare S, Henttu PM, Parker MG. Molecular determinants of the estrogen receptor-coactivator interface. *Mol Cell Biol* 1999;19(5):3895–903.

[28] Brzozowski AM, Pike AC, Dauter Z, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 1997;389(6652): 753–8.

[29] Shiau AK, Barstad D, Loria PM, et al. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* 1998;95(7):927–37.

[30] Pike AC, Brzozowski AM, Hubbard RE, et al. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *EMBO* J 1999;18(17):4608–18.

[31] McDonnell DP, Clemm DL, Hermann T, Goldman ME, Pike JW. Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens. *Mol Endocrinol* 1995;9(6):659–69.

[32] Paige LA, Christensen DJ, Gron H. et al. Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. *Proc Natl Acad Sci USA* 1999;96(7):3999–4004.

[33] Katzenellenbogen JA, O'Malley BW, Katzenellenbogen BS. Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Mol Endocrinol* 1996;10(2):119–31.

[34] Galien R, Garcia T. Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kappaB site. *Nucleic Acids Res* 1997;25(12):2424–9.

[35] Montano MM, Jaiswal AK, Katzenellenbogen BS. Transcriptional regulation of the human quinone reductase gene by antiestrogen-liganded estrogen receptor-alpha and estrogen receptor-beta. *J Biol Chem* 1998;273(39):25443–9.

[36] Webb P, Nguyen P, Valentine C, et al. The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions. *Mol Endocrinol* 1999;13(10):1672–85.

[37] Qin C, Singh P, Safe S. Transcriptional activation of insulin-like growth factor-binding protein-4 by 17 beta-estradiol in MCF-7 cells: role of estrogen receptor-SP1 complexes. *Endocrinology* 1999;140(6):2501–8.

[38] Saville B, Wormke M, Wang F, et al. Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements. *J Biol Chem* 2000;275(8):5379–87.

[39] Paech K, Webb P, Kuiper GGJM, et al. Differential ligand activation of estrogen receptors ER alpha and ER beta at AP1 sites. *Science* 1997;277(5331):1508–10.

[40] Power RF, Mani SK, Codina J, Conneely OM, O'Malley BW. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* 1991;254(5038):1636–9.

[41] Chalbos D, Philips A, Galtier F, Rochefort H. Synthetic antiestrogens modulate induction of pS2 and cathepsin-D messenger ribonucleic acid by growth factors and 3´,5´-monophosphate in MCF7 cells. *Endocrinology* 1993; 133(2):571–6.

[42] Kato S, Endoh H, Masuhiro Y, et al. Activation of the estrogen receptor through phosphorylation by mitogen- activated protein kinase. *Science* 1995;270(5241):1491–94.

[43] Bunone G, Briand PA, Miksicek RJ, Picard D. Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO* J 1996;15(9):2174–83.

[44] Ma ZQ, Santagati S, Patrone C, Pollio G, Vegeto E, Maggi A. Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3. *Mol Endocrinol* 1994;8(7): 910–8.

[45] Ignar-Trowbridge DM, Nelson KG, Bidwell MC, Curtis SW, Washburn TF, McLachlan JA, Korach KS. Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor. *Proc Natl Acad Sci USA* 1992;89(10):4658–62.

[46] Smith CL, Conneely OM, O'Malley BW. Modulation of the ligand-independent activation of the human estrogen receptor by hormone and antihormone. *Proc Natl Acad Sci USA* 1993;90(13):6120–4.

[47] Patrone C, Gianazza E, Santagati S, Agrati P, Maggi A. Divergent pathways regulate ligand-independent activation of ER alpha in SK-N-BE neuroblastoma and COS-1 renal carcinoma cells. *Mol Endocrinol* 1998;12(6):835–41.

[48] Cenni B, Picard D. Ligand-independent activation of steroid receptors: new roles for old players. *Trends Endocrinol Metab* 1999;10(41):41–6.

[49] Migliaccio A, Di Domenico M, Castoria G, et al. Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. *EMBO* J 1996;15(6):1292–300.

[50] Collins P, Webb C. Estrogen hits the surface. Nat Med 1999;5(10):1130–1.

[51] Subbiah MT. Mechanism of cardioprotection by estrogens. *Proc Soc Exp Biol Med* 1998;217:23–9.

[52] Moosmann B, Behl C. The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic properties. *Proc Nat Acad Sci USA* 1999;96(16):8867–72.

[53] Behl C. Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog Neurobiol* 1999;57(3):301–23.

[54] Heck S, Lezoualc'h F, Engert S, Behl C. Insulin-like growth factor-1-mediated neuroprotection against oxidative stress is associated with activation of nuclear factor kappaB. *J Biol Chem* 1999;274(14):9828–35.

[55] Behl C, Trapp T, Skutella T, Holsboer F. Protection against oxidative stress-induced neuronal cell death-a novel role for RU486. *Eur J Neurosci* 1997;9(5):912–20.

[56] Montano MM, Katzenellenbogen BS. The quinone reductase gene: a unique estrogen receptor-regulated gene that is activated by antiestrogens. *Proc Natl Acad Sci USA* 1997;94(6):2581–6.

[57] Short TH, Rothschild MF, Southwood OI, et al. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *J Anim Sci* 1997;75(12):3138–42.

[58] Andersen TI, Heimdal KR, Skrede M, Tveit K, Berg K, Borresen AL. Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. *Hum Genet* 1994;94(6):665–70.

[59] Mizunuma H, Hosoi T, Okano H, et al. Estrogen receptor gene polymorphism and bone mineral density at the lumbar spine of premenopausal and postmenopausal women. *Bone* 1997;21(5):379–83.

[60] Lehrer S, Rabin J, Kalir T, Schachter BS. Estrogen receptor variant and hypertension in women. *Hypertension* 1993;21(4):439–41.

[61] Schachter BS, Lehrer S. Re: "Risk of miscarriage and a common variant of the estrogen receptor gene". *Am J Epidemiol* 1994;140(12):1144–5.

[62] Lehrer S, Rabin J, Stone J, Berkowitz GS. Association of an estrogen receptor variant with increased height in women. *Horm Metab Res* 1994;26(10):486–8.

[63] MacGillivray MH, Morishima A, Conte F, Grumbach M, Smith EP. Pediatric endocrinology update: an overview. The essential roles of estrogens in pubertal growth, epiphyseal fusion and bone turnover: lessons from mutations in the genes for aromatase and the estrogen receptor. *Horm Res* 1998;49(3)(Suppl 1):2–8.

[64] Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 1999;20:358–417.

[65] Cunha GR, Young P, Hom YK, Cooke PS, Taylor JA, Lubahn DB. Elucidation of a role for stromal steroid hormone receptors in mammary gland growth and development using tissue recombinants. *J Mammary Gland Biol Neoplasia* 1997;2(4):393–402.

[66] Leygue E, Dotzlaw H, Watson PH, Murphy LC. Expression of estrogen receptor beta1, beta2, and beta5 messenger RNAs in human breast tissue. *Cancer Res* 1999;59(6):1175–9.

[67] Saji S, Jensen EV, Nilsson S, Rylander T, Warner M, Gustafsson J-Å. Estrogen receptors alpha and beta in the rodent mammary gland. *Proc Natl Acad Sci USA* 2000;97(1):337–42.

[68] Speirs V, Parkes AT, Kerin MJ, et al. Coexpression of estrogen receptor-alpha and -beta: poor prognostic factors in human breast cancer? *Cancer Res* 1999;59(3):525–8.

[69] Fisher CR, Graves KH, Parlow AF, Simpson ER. Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. *Proc Natl Acad Sci USA* 1998;95(12):6965–70.

[70] Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 1994;331(16):1056–61.

[71] Robertson KM, O'Donnell L, Jones ME, et al. Impairment of spermatogenesis in mice lacking a functional aromatase (cyp 19) gene. *Proc Natl Acad Sci USA* 1999;96(14):7986–91.

[72] Couse JF, Bunch DO, Lindzey J, Schomberg DW, Korach KS. Prevention of the polycystic ovarian phenotype and characterization of ovulatory capacity in the estrogen receptor-alpha knockout mouse. *Endocrinology* 1999;140(12):5855–65.

[73] Mäkelä S, Strauss L, Kuiper G, et al. Differential expression of estrogen receptors alpha and beta in adult rat accessory sex glands and lower urinary tract [corrected and republished from *Mol Cell Endocrinol* 2000;164(1-2):109–16. Mol Cell Endocrinol 2000;170(1-2):217–29].

[74] Chang WY, Prins GS. Estrogen receptor-beta: implications for the prostate gland. *Prostate* 1999; 40(2):115–24.

[75] Weihua Z, Mäkelä S, Andersson LC, et al. A role for estrogen receptor beta in the regulation of growth of the ventral prostate. *Proc Natl Acad Sci USA* 2001;98(11):6330–5.

[76] Prins GS, Marmer M, Woodham C, et al. Estrogen receptor-beta messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats. *Endocrinology* 1998;139(3):874–83.

[77] Lindsay R, Hart DM, Aitken JM, MacDonald EB, Anderson JB, Clarke AC. Long-term prevention of postmenopausal osteoporosis by oestrogen. Evidence for an increased bone mass after delayed onset of oestrogen treatment. *Lancet* 1976;1(7968):1038–41.

[78] Cauley JA, Seeley DG, Ensrud K, Ettinger B, Black D, Cummings SR. Estrogen replacement therapy and fractures in older women. Study of Osteoporotic Fractures Research Group. *Ann Intern Med* 1995;122(1):9–16.

[79] Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC. Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. *J Clin Invest* 1997;100(7):1755–9.

[80] Barrett-Connor E, Mueller JE, von Muhlen DG, Laughlin GA, Schneider DL, Sartoris DJ. Low levels of estradiol are associated with vertebral fractures in older men, but not women: the Rancho Bernardo study. *J Clin Endocrinol Metab* 2000;85(1):219–23.

[81] Komm BS, Terpening CM, Benz DJ, et al. Estrogen binding, receptor mRNA, and biological response in osteoblast-like osteosarcoma cells. *Science* 1988;241(4861):81–4.

[82] Eriksen EF, Colvard DS, Berg NJ, et al. Evidence of estrogen receptors in normal human osteoblast-like cells. *Science* 1988;241(4861):84–6.

[83] Oursler MJ, Pederson L, Fitzpatrick L, Riggs BL, Spelsberg T. Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. *Proc Natl Acad Sci USA* 1994;91(12):5227–31.

[84] Onoe Y, Miyaura C, Ohta H, Nozawa S, Suda T. Expression of estrogen receptor β in rat bone. *Endocrinology* 1997;138(10):4509–12.

[85] Arts J, Kuiper GGJM, Janssen JM, et al. Differential expression of estrogen receptors alpha and beta mRNA during differentiation of human osteoblast SV-HFO cells. *Endocrinology* 1997;138(11):5067–70.

[86] Chen MM, Yeh JK, Aloia JF. Effect of ovariectomy on cancellous bone in the hypophysectomized rat. *J Bone Miner Res* 1995;10(9):1334–42.

[87] Friend KE, Hartman ML, Pezzoli SS, Clasey JL, Thorner MO. Both oral and transdermal estrogen increase growth hormone release in post-menopausal women–a clinical research center study. *J Clin Endocrin Metab* 1996;81(6):2250–6.

[88] Sandstedt J, Tornell J, Norjavaara E, Isaksson OG, Ohlsson C. Elevated levels of growth hormone increase bone mineral content in normal young mice, but not in ovariectomized mice. *Endocrinology* 1996;137(8):3368–74.

[89] Scillitani A, Chiodini I, Carnevale V, et al. Skeletal involvement in female acromegalic subjects: the effects of growth hormone excess in amenorrheal and menstruating patients. *J Bone Miner Res* 1997;12(10):1729–36.

[90] Slootweg MC, Swolin D, Netelenbos JC, Isaksson OG, Ohlsson C. Estrogen enhances growth hormone receptor expression and growth hormone action in rat osteosarcoma cells and human osteoblast-like cells. *J Endocrinol* 1997;155(1):159–64.

[91] Bryant HU, Glasebrook AL, Yang NN, Sato M. An estrogen receptor basis for raloxifene action in bone. *J Steroid Biochem Mol Biol* 1999;69(1-6):37–44.

[92] Ralston SH, Russell RG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. *J Bone Miner Res* 1990;5(9):983–8.

[93] Kimble RB, Srivastava S, Ross FP, Matayoshi A, Pacifici R. Estrogen deficiency increases the ability of stromal cells to support murine osteoclastogenesis via an interleukin-1 and tumor necrosis factor-mediated stimulation of macrophage colony-stimulating factor production. *J Biol Chem* 1996;271(46):28890–7.

[94] Jilka RL. Cytokines, bone remodeling, and estrogen deficiency: a 1998 update. *Bone* 1998;23(2):75–81.

[95] Spelsberg TC, Subramaniam M, Riggs BL, Khosla S. The actions and interactions of sex steroids and growth factors/cytokines on the skeleton. *Mol Endocrinol* 1999;13(6):819–28.

[96] Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res* 2000;15(1):2–12.

[97] Windahl SH, Vidal O, Andersson G, Gustafsson J-Å, Ohlsson C. Increased cortical bone mineral content but unchanged trabecular bone mineral density in female ERbeta(-/-) mice. *J Clin Invest* 1999;104(7):895–901.

[98] Lee PA, Witchel SF. The influence of estrogen on growth. Curr Opin Pediatr 1997;9(4):431–6.

[99] Klein KO, Martha PM Jr, Blizzard RM, Herbst T, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. II. Estrogen levels as determined by an ultrasensitive bioassay. *J Clin Endocrinol Metab* 1996;81(9):3203–7.

[100] Grodstein F, Stampfer MJ, Colditz GA, et al. Postmenopausal hormone therapy and mortality. *N Engl J Med* 1997;336(25):1769–75.

[101] Nathan L, Chaudhuri G. Estrogens and atherosclerosis. *Annu Rev Pharmacol Toxicol* 1997;37:477–515.

[102] Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340(23):1801–11.

[103] Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 1998;280(7):605–13.

[104] Register TC, Adams MR. Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. *J Steroid Biochem Mol Biol* 1998;64(3-4):187–91.

[105] Grohé C, Kahlert S, Lobbert K, Vetter H. Expression of oestrogen receptor alpha and beta in rat heart: role of local oestrogen synthesis. *J Endocrinol* 1998;156(2):R1–7.

[106] Grohé C, Kahlert S, Lobbert K, et al. Cardiac myocytes and fibroblasts contain functional estrogen receptors. *FEBS Lett* 1997;416(1):107–12.

[107] Foegh ML, Ramwell PW. Cardiovascular effects of estrogen: implications of the discovery of the estrogen receptor subtype beta. *Curr Opin Nephrol Hypertens* 1998;7(1):83–9.

[108] Valverde MA, Rojas P, Amigo J, et al. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. *Science* 1999;285(5435):1929–31.

[109] Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW. Estrogen receptor beta mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest* 1999;103(3):401–6.

[110] Mikkola T, Viinikka L, Ylikorkala O. Estrogen and postmenopausal estrogen/progestin therapy: effect on endothelium-dependent prostacyclin, nitric oxide and endothelin-1 production. *Eur J Obstet Gynecol Reprod Biol* 1998;79(1):75–82.

[111] Nickenig G, Baumer AT, Grohe C, et al. Estrogen modulates AT1 receptor gene expression in vitro and in vivo. *Circulation* 1998;97(22):2197–201.

[112] Akishita M, Ouchi Y, Miyoshi H, et al. Estrogen inhibits cuff-induced intimal thickening of rat femoral artery: effects on migration and proliferation of vascular smooth muscle cells. *Atherosclerosis* 1997;130(1-2):1–10.

[113] Scheuer J, Malhotra A, Schaible TF, Capasso J. Effects of gonadectomy and hormonal replacement on rat hearts. *Circ Res* 1987;61(1):12–9.

[114] Malhotra A, Buttrick P, Scheuer J. Effects of sex hormones on development of physiological and pathological cardiac hypertrophy in male and female rats. *Am J Physiol* 1990;259(3 Pt 2):H866–71.

[115] Marcus R, Krause L, Weder AB, Dominguez-Meja A, Schork NJ, Julius S. Sex-specific determinants of increased left ventricular mass in the Tecumseh Blood Pressure Study. *Circulation* 1994;90(2):928–36.

[116] Gardin JM, Wagenknecht LE, Anton-Culver H, et al. Relationship of cardiovascular risk factors to echocardiographic left ventricular mass in healthy young black and white adult men and women. The CARDIA study. Coronary Artery Risk Development in Young Adults. *Circulation* 1995;92(3):380–7.

[117] Collins P, Rosano GM, Sarrel PM, et al. 17 beta-estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation* 1995;92:24–30.

[118] Sudhir K, Chou TM, Messina LM, et al. Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene. *Lancet* 1997;349(9059):1146–7.

[119] Sudhir K, Chou TM, Chatterjee K, et al. Premature coronary artery disease associated with a disruptive mutation in the estrogen receptor gene in a man. *Circulation* 1997;96(10):3774–7.

[120] Brinton EA. Oral estrogen replacement therapy in postmenopausal women selectively raises levels and production rates of lipoprotein A-I and lowers hepatic lipase activity without lowering the fractional catabolic rate. *Arterioscler Thromb Vasc Biol* 1996;16(3):431–40.

[121] Zysow BR, Kauser K, Lawn RM, Rubanyi GM. Effects of estrus cycle, ovariectomy, and treatment with estrogen, tamoxifen, and progesterone on apolipoprotein(a) gene expression in transgenic mice. *Arterioscler Thromb Vasc Biol* 1997;17(9):1741–5.

[122] Schunkert H, Danser AH, Hense HW, Derkx FH, Kurzinger S, Riegger GA. Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women. *Circulation* 1997;95(1):39–45.

[123] Seishima M, Bisgaier CL, Davies SL, Glickman RM. Regulation of hepatic apolipoprotein synthesis in the 17 alpha-ethinyl estradiol-treated rat. *J Lipid Res* 1991;32(6):941–51.

[124] Maes M, De Hertogh R, Watrin-Granger P, Ketelslegers JM. Ontogeny of liver somatotropic and lactogenic binding sites in male and female rats. *Endocrinology* 1983;113(4):1325–32.

[125] Gabrielsson BG, Carmignac DF, Flavell DM, Robinson IC. Steroid regulation of growth hormone (GH) receptor and GH-binding protein messenger ribonucleic acids in the rat. *Endocrinology* 1995;136(1):209–17.

[126] Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of the estrogen receptors alpha and beta. *Endocrinology* 1997;138(3):863–70.

[127] Clinton SK, Libby P. Cytokines and growth factors in atherogenesis. *Arch Pathol Lab Med* 1992;116(12):1292–1300.

[128] St Clair RW. Effects of estrogens on macrophage foam cells: a potential target for the protective effects of estrogen on atherosclerosis. *Curr Opin Lipidol* 1997;8(5):281–6.

[129] Libby P, Schwartz D, Brogi E, Tanaka H, Clinton SK. A cascade model for restenosis. A special case of atherosclerosis progression. *Circulation* 1992;86(Suppl 6):III47–52.

[130] Gulshan S, McCruden AB, Stimson WH. Oestrogen receptors in macrophages. *Scand J Immunol* 1990;31(6):691–7.

[131] Huber LA, Scheffler E, Poll T, Ziegler R, Dresel HA. 17 beta-estradiol inhibits LDL oxidation and cholesteryl ester formation in cultured macrophages. *Free Radic Res Commun* 1990;8(3):167–73.

[132] Sulistiyani, St Clair RW. Effect of 17 beta-estradiol on metabolism of acetylated low-density lipoprotein by THP-1 macrophages in culture. *Arterioscler Thromb Vasc Biol* 1997;17(9):1691–1700.

[133] Shanker G, Sorci-Thomas M, Adams MR. Estrogen modulates the expression of tumor necrosis factor alpha mRNA in phorbol ester-stimulated human monocytic THP-1 cells. *Lymphokine Cytokine Res* 1994;13(6):377–82.

[134] Srivastava S, Weitzmann MN, Cenci S, Ross FP, Adler S, Pacifici R. Estrogen decreases TNF gene expression by blocking JNK activity and the resulting production of c-Jun and JunD. *J Clin Invest* 1999;104(4):503–13.

[135] McEwen BS, Alves SE. Estrogen actions in the central nervous system. *Endocr Rev* 1999;20(3):279–307.

[136] Bethea CL, Pecins-Thompson M, Schutzer WE, Gundlah C, Lu ZN. Ovarian steroids and serotonin neural function. *Mol Neurobiol* 1998;18(8):87–123.

[137] Pecins-Thompson M, Brown NA, Kohama SG, Bethea CL. Ovarian steroid regulation of tryptophan hydroxylase mRNA expression in rhesus macaques. *J Neurosci* 1996;16(21):7021–9.

[138] Pecins-Thompson M, Brown NA, Bethea CL. Regulation of serotonin re-uptake transporter mRNA expression by ovarian steroids in rhesus macaques. *Brain Res Mol Brain Res* 1998;53(1–2):120–9.

[139] Pecins-Thompson M, Bethea CL. Ovarian steroid regulation of serotonin-1A autoreceptor messenger RNA expression in the dorsal raphe of rhesus macaques. *Neuroscience* 1999;89(1):267–77.

[140] Lakoski JM. Estrogen-induced modulation of serotonin 5-HT1A mediated responses in the dorsal raphe nucleus (DRN) (abstract 94.6). *Pharmacologist* 1988;30:A126.

[141] Biegon A, McEwen BS. Modulation by estradiol of serotonin receptors in brain. *J Neurosci* 1982;2:199–205.

[142] Lucas JJ, Hen R. New players in the 5-HT receptor field: genes and knockouts. *Trends Pharmacol Sci* 1995;16(7):246–52.

[143] Sumner BE, Fink G. Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype messenger-RNA expression in female rat brain. *Mol Cell Neurosci* 1993;4:83–92.

[144] Sumner BE, Fink G. The density of 5-hydroxytryptamine2A receptors in forebrain is increased at pro-oestrus in intact female rats. *Neurosci Lett* 1997;234(1):7–10.

[145] Cyr M, Bossé R, Di Paolo T. Gonadal hormones modulate 5-hydroxytryptamine 2A receptors: emphasis on the rat frontal cortex. *Neuroscience* 1998;83(3):829–36.

[146] Sumner BE, Fink G. Testosterone as well as estrogen increases serotonin^{2A} receptor mRNA and binding sites in the male rat brain. *Brain Res Mol Brain Res* 1998;59(2):205–14.

[147] Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol* 1997;388(4):507–25.

[148] Shughrue PJ, Lane MV, Merchenthaler I. Biologically active estrogen receptor beta: evidence from in vivo autoradiographic studies with estrogen receptor alpha-knockout mice. *Endocrinology* 1999;140(6):2613–20.

[149] Di Paolo T. Modulation of brain dopamine transmission by sex steroids. *Rev Neurosci* 1994;5(1):27–41.

[150] Saunders-Pullman R, Gordon-Elliott J, Parides M, Fahn S, Saunders HR, Bressman S. The effect of estrogen replacement on early Parkinson's disease. *Neurology* 1999;52(7):1417–21.

[151] Crowley WR, O´Donohue TL, Jacobowitz DM. Changes incatecholamine content in discrete brain nuclei during the estrous cycle of the rat. *Brain Res* 1978;147(2):315–26.

[152] Di Paolo T, Rouillard C, Bedard P. 17 beta-estradiol at a physiological dose acutely increases dopamine turnover in the rat brain. *Eur J Pharmacol* 1985;117(2):197–203.

[153] Becker JB. Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. *Synapse* 1990;5(2):157–64.

[154] Bossé R, Rivest R, Di Paolo T. Ovariectomy and estradiol treatment affect the dopamine transporter and its gene expression in the rat brain. *Brain Res Mol Brain Res* 1997;46(1-2):343–6.

[155] Roy EJ, Buyer DR, Licari VA. Estradiol in the striatum: effects on behavior and dopamine receptors but no evidence for membrane steroid receptors. *Brain Res Bull* 1990;25(2):221–7.

[156] Cramer OM, Parker CR Jr, Porter JC. Estrogen inhibition of dopamine release into hypophysial portal blood. *Endocrinology* 1979;104(2):419–22.

[157] Kuiper GGJM, Shughrue PJ, Merchenthaler I, Gustafsson J-Å. The estrogen receptor beta subtype: a novel mediator of estrogen action in neuroendocrine systems. *Front Neuroendocrinol* 1998;19(4):253–86.

[158] Österlund MK, Gustafsson J-Å, Keller E, Hurd YL. Estrogen receptor beta (ERbeta) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to the ERalpha mRNA. *J Clin Endocrinol Metab* 2000;85(10):3840–6.

[159] Alves SE, Lopez V, McEwen BS, Weiland NG. Differential colocalization of estrogen receptor beta (ERbeta) with oxytocin and vasopressin in the paraventricular and supraoptic nuclei of the female rat brain: an immunocytochemical study. *Proc Natl Acad Sci USA* 1998;95(6):3281–6.

[160] Hrabovszky E, Kallo I, Hajszan T, Shughrue PJ, Merchenthaler I, Liposits Z. Expression of estrogen receptor-beta messenger ribonucleic acid in oxytocin and vasopressin neurons of the rat supraoptic and paraventricular nuclei. *Endocrinology* 1998;139(5):2600–4.

[161] Zorrilla R, Simard J, Rheaume E, Labrie F, Pelletier G. Multihormonal control of pre-pro-somatostatin mRNA levels in the periventricular nucleus of the male and female rat hypothalamus. *Neuroendocrinology* 1990;52(5):527–36.

[162] Shen ES, Hardenburg JL, Meade EH, Arey BJ, Merchenthaler I, Lopez FJ. Estradiol induces galanin gene expression in the pituitary of the mouse in an estrogen receptor alpha-dependent manner. *Endocrinology* 1999;140(6):2628–31.

[163] Grattan DR, Rocca MS, Strauss KI, Sagrillo CA, Selmanoff M, McCarthy MM. GABAergic neuronal activity and mRNA levels for both forms of glutamic acid decarboxylase (GAD65 and GAD67) are reduced in the diagional band of Broca during the afternoon of proestrus. *Brain Res* 1996;733(1):46–55.

[164] Woolley C, McEwen BS. Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor dependent mechanism. *J Neurosci* 1994;14(12):7680–7.

[165] Diano S, Naftolin F, Horvath TL. Gonadal steroids target AMPA glutamate receptor-containing neurons in the rat hypothalamus, septum and amygdala: a morphological and biochemical study. *Endocrinology* 1997;138(2):778–89.

[166] Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (ERalpha and ERbeta) throughout the rat brain: anatomical evidence of distinct roles of each subtype. *J Neurobiol* 1998;36(3):357–78.

[167] Shughrue PJ, Lubahn DB, Negro-Vilar A, Korach KS, Merchenthaler I. Responses in the brain of estrogen receptor alpha-disrupted mice. *Proc Natl Acad Sci USA* 1997;94(20):11008–12.

[168] Shughrue PJ, Lane MV, Merchenthaler I. Biologically active estrogen receptor-beta: evidence from in vivo autoradiographic studies with estrogen receptor alpha-knockout mice. *Endocrinology* 1999;140(6):2613–20.

[169] Renier MA, Vereecken A, Buytaert P. Inhibins, activins and follistatins: a review of complex regulators of the reproductive system. *Eur J Contracept Reprod Health Care* 1998;3(3):129–35.

[170] Lockwood GM, Muttukrishna S, Ledger WL. Inhibins and activins in human ovulation, conception and pregnancy. *Hum Reprod Update* 1998;4(3):284–95.

[171] Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF Jr. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. *J Clin Endocrinol Metab* 1991;73(3):621–8.

[172] Bagatell CJ, Dahl KD, Bremner WJ. The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. *J Androl* 1994;15(1):15–21.

[173] Carani C, Qin K, Simoni M, et al. Effect of testosterone and estradiol in a man with aromatase deficiency. *N Engl J Med* 1997;337(2):91–5.

[174] Fekete CS, Strutton PH, Cagampang FRA, et al. Estrogen receptor immunoreactivity is present in the majority of central histaminergic neurons: evidence for a new neuroendocrine pathway associated with luteinizing hormone-releasing hormone-synthesizing neurons in rats and humans. *Endocrinology* 1999;140(9):4335–41.

[175] Mahesh VB, Brann DW. Regulation of the preovulatory gonadotropin surge by endogenous steroids. *Steroids* 1998;63(12):616–29.

[176] Pinkerton JV, Santen R. Alternatives to the use of estrogen in postmenopausal women. *Endocr Rev* 1999;20(3):308–20.

[177] Bryant HU, Dere WH. Selective estrogen receptor modulators: an alternative to hormone replacement therapy. *Proc Soc Exp Biol Med* 1998;217(1):45–52.

[178] Cosman F, Lindsay R. Selective estrogen receptor modulators: clinical spectrum. *Endocr Rev* 1999;20(3):418–34.

[179] Sun J, Meyers MJ, Fink BE, Rajendran R, Katzenellenbogen JA, Katzenellenbogen BS. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-alpha or estrogen receptor-beta. *Endocrinology* 1999;140(2):800–4.